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ТЕОРИЯ И ПРАКТИКА ПЕРЕРАБОТКИ МЯСА

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ЦЕЛИ И ЗАДАЧИ ЖУРНАЛА

Приоритетной целью Журнала «Теория и практика переработки мяса» является распространение в мировом научном сообществе трудов по науке о мясе ученых научных центров, научно-исследовательских институтов и высших учебных заведений из России и стран СНГ, повышение уровня присутствия достижений представляемой ими науки на международной арене, знакомство Российских ученых с исследованиями за рубежом, освещение результатов перспективных направлений научно-исследовательской деятельности в мясной и птицеперерабатывающей промышленности.

К публикации в журнале приглашаются как отечественные, так и зарубежные ученые и специалисты.

Важнейшими задачами журнала являются: обобщение научных и практических достижений в области науки о мясе, повышения научной и практической квалификации как научных работников, так и представителей промышленности.

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The top priority goal of the Journal "Teoriâ i praktika pererabotki mâsa" (Theory and practice of meat processing) is to distribute in the world scientific community the results of the research in the field of meat science performed by the scientists from scientific centers, scientific-research institutes and institutions of higher education from Russia and the CIS countries, increase the level of presentation of the achievements of the respective science in the international arena, inform the Russian scientists about the research carried out abroad, highlight the results of the prospect directions of the research activities in the meat and poultry processing industries.

Both Russian and foreign scientists and experts are invited for publication in the journal.

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AN EFFECT OF ANTI-STRESS FEED ADDITIVES ON BROILER PRODUCTIVITY AND MEAT QUALITY

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Keywords: *anti-stress feed additive, broiler chickens, chemical composition, average daily gain*

Abstract
The paper presents the study on an impact of feed additives Peak anti-stress and SPAO (SPAO-complex) with different lithium content on meat productivity and meat quality of broiler chickens. The feed additives exert a pronounced metabolic effect, have adaptogen properties and allow forming a mechanism that facilitates compensation of the expenditure of the body, which significantly increases upon stress development. It was established that the average daily gain of the broiler chickens increased by 1.8% and 4.3% on the background of using SPAO-complex and Peak anti-stress, respectively, compared to the broiler chickens that did not receive the feed additives in the daily diet. It was shown that addition of feed additives with lithium into a diet led to an improvement of the indices of broiler meat productivity and meat quality: a level of yield of the carcasses of the 1st category increased up to 56.2–79.1%, high organoleptic indices of meat were ensured, the protein content in white and red chicken meat increased and functional-technological properties of minced meat improved. The use of feed additives ensured profitability of industrial poultry production; the highest indices of profitability were established upon introduction of the feed additive Peak anti-stress into a diet — up to 8.67 rubles per each ruble of expenses. The obtained results of the study should be taken into consideration in the technological processes when raising broiler chickens.

Introduction

According to the Food Security Doctrine of the Russian Federation adopted by the Executive Order of the President of the Russian Federation of January 21, 2020, the agro-industrial complex of the country faces a task of creation, reconstruction and development of modern productions regarding the output of feed additives for animals to increase meat raw material production including poultry. When forming poultry meat quality, an important role is given to prevention of stressful situations in the conditions of industrial poultry production by using feed additives that mitigate an effect of stress factors.

Several authors [1] notice an important role of antioxidants in the composition of a diet. These antioxidants include the natural bioflavonoid dihydroquercetin and arabinogalactan — a water-soluble polysaccharide of plant origin. Their addition to the combined feed for broilers affects the lipid metabolism, which is characterized by a decrease in the fat content in pectoral muscles, and also facilitates a decrease in peroxide compounds in broiler meat due to the low content of abdominal fat in a carcass, and in a dose of 3.6 g/ton increases broiler liveweight by 3.97%, improves feed conversion.

The use of the feed additive HydroLactiv and antioxidant Epophen in combined feed and their conjunct influence have a positive effect on productivity and meat quality of Cobb broiler chickens. The authors [2] note that the average daily liveweight gain increases by 11.0%, the efficiency of digestion and utilization of dietary nutrients is stimulated, which increases feed conversion into meat products — feed consumption per 1 kg of the liveweight gain decreases by 9.9%. According to the data of controlled slaughter, balanced feeding improves broiler meat quality. For example, pre-slaughter weight of broiler chickens increases by 10.7%, the weight of an eviscerated carcass by 368.4 g or 15.89%, slaughter yield of broilers by 0.85%. The chemical composition of broiler meat changes by 8.49% in terms of the dry matter content and the protein content in broiler meat changes by 0.91%.

The anti-stress additive Acti'z Vitamins B in an effervescent form for egg-laying hens contains nicotinamide, pantothenic acid, vitamins B1, B2, B6, folic acid, D- biotin and vitamin B12. It is added during the period of intensive egg-laying and the peak of productivity of hens from the parent population and according to some authors [3] facilitates an improvement in the metabolic processes, poultry productivity and quality of produced young birds.

It was established that the antioxidant of the new generation Bisphenol-5 had a positive effect on the hematological and biochemical parameters of blood and growth of birds. For example, upon addition of the antioxidant into complete feed at a dose of 0.0002%, 0,0004%, 0.0008% and 0.0015% of the daily ration, an increase in hemoglobin by 2.6–21.6%, red blood cells by 5.1–47.6%, protein by 15.7%, glucose by 7.8%, calcium and inorganic phosphorus by 6.1% and 6.5%, respectively, was observed in the chickens of the experimental groups. The activity of enzymes taking part in digestion of feed components increased by 14.7 and 8.3%, which indicates a positive effect of the additive on the protein, carbohydrate and mineral metabolism, as well as the enzymatic activity [4].

The study [5] describes a positive role of L-carnitine and preparations on its basis on the lipid metabolism in broiler chickens. L-carnitine is important in transportation of fatty acids into mitochondria ensuring their oxidation. According

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to the data of the authors, the preparation Strolitin, which active ingredient is L-carnitine and accessory substances are magnesium sulfate, sorbitol and distilled water, has the anabolic, antihypoxic and antithyroid action, stimulates the regenerative activity of tissues, normalizes the metabolic processes, prevents osteoporosis and activates fat metabolism. When receiving the preparation from the age of 1–5 days and from 21–25 day, broiler chickens show a 6.95% and 6.09% increase in liveweight by the end of raising, a 6.3% and 5.35% decrease in feed consumption per gain, respectively; in addition, a positive effect on liveability of the population was observed.

Prokhorova Yu.V. et al. (2016) reported that feed additives with microelements, in particular, the preparation Selemagum-O (a water soluble complex of selenium and vitamin E) had a positive effect on the body of broiler chickens; when using the preparation from the first days of life of broiler chickens at a dose of 1 ml/100 l water, the indices of gain and liveability improved on average by 3.5% [6].

Zinc has been actively used. It is a component of more than 200 metalloenzymes, affects the cell growth and division, skin condition, plumage, osteogenesis, wound healing, reproductive function, immune system, cellular respiration, brain development, formation of behavioral reflexes and so on. Skopichev V. G. et al. [7] noted that zinc deficiency caused stunting, retardation of the development, reduction in egg production, disorder of eggshell formation.

Among the agents that affect the nervous system and are used for stress prevention in animal husbandry are lithium salts. According to the data [8] «...lithium preparations are psychotropic pharmaceutical products from the group of mood stabilizers. Lithium is an alkali metal; therefore, it is used in medicine and veterinary medicine in a form of salts — carbonate, oxybutirate, nicotinate, succinate, chloride, sulfate and citrate, and their use is conditioned by the specific mechanism of action, which is based on an effect of the lithium ion on different divisions of the nervous system...»

Fisinin V. I. et al. [9] note that «... the use of lithium carbonate at a dose of 15 mg/kg body weight of broilers facilitated an increase in liveability and average daily increase in the population. Lithium carbonate at a dose of 15 mg/kg body weight of broilers is expedient to use in broiler production enterprises as it facilitates acceleration of growth, an increase in liveweight gain, liveability of the population...»

Bachinskaya V. M. [10] recommends to «...use lithium citrate in hens of the parent flock and broiler chickens two days before testing stress sensitivity, on the day of testing and during two days after the turpentine test. Administration of lithium citrate does not influence formation of the local adaptive syndrome at the point of injection of the irritating substance...»

Effectiveness of lithium salt addition into a diet as a means of stress prevention is confirmed in [11,12]. The use of feed additives with the probiotic [13,14] and antioxidant [5] properties facilitates formation of meat productivity and production of high-quality meat.

To reduce the unfavorable effect of heat stress or stress caused by vaccination, as well as antibiotics or anticoccidial agents, it is recommended to use the feed additive Betamint, which consists of biologically active substances: vitamin C, betaine, menthol. Betamint exerts a rapid and durable effect in the control of heat stress; the preparation is given with water at a dose of 1 l/ton over three days before and after the stress factor [15].

According to the data [16,17], «...the use of the anti-stress preparations Vitaminoacid and Magic Antistress Mix on the background of industrial stress factors ensures superiority over the control in terms of liveweight on average by 5.15 and 2.17% in replacement pullets, by 0.73 and 0.73% in laying hens, by 4.46 and 1.06 in replacement cockerels, and by 1.33 and 3.04% in roosters; the uniformity of the population increased by 1.08 and 5.49% in pullets, by 6.95 and 9.30% in cockerels and by 2.90 and 8.13% in laying hens, respectively; the uniformity of roosters in the second experimental group increased by 7.41%. Vitaminoacid and Magic Antistress Mix facilitated an increase in liveability of the population of replacement pullets by 0.45 and 0.52%, cockerels by 0.94 and 1.38%, a reduction of feed expenditure by young birds by 0.33 and 1.33%, liveability of laying hens by 0.1 and 0.41%, roosters by 0.57 and 1.14%, respectively. They had a positive effect on the growth and metabolic processes in the body of young poultry.

Engashev S. V. et al. [18] proposed a method for reduction of heat stress in broilers by adding the antioxidant with the 3-oxypyridine structure into a diet, which allowed increasing liveability of the poultry population in the conditions of heat stress by 1.04% and increasing the average daily gain by 7.7%.

A group of authors under the leadership of R. Kh. Gadzaonov [19] developed a means for stress prevention in poultry production using pharmacological preparations from the group of tranquilizers phenazepam and the Eleutherococci extract, which showed high liveability of poultry population (95–90%) and additional gains. However, the United Nations Commission on Narcotic Drugs placed phenazepam into a list of substances subjected to special control; the use of the proposed scheme determines a necessity of individual administration of preparations, which makes their use in industrial poultry production more difficult.

The use of agents from different pharmacological groups influencing formation of the stress reaction and its strength for stress prevention in poultry production determines the course of adaptive processes in the body of birds. The promising directions are the use of complex preparations and preparations based on lithium salts, which have multifaceted mechanisms of the pharmacological action and low toxicity as stress protectors; and antioxidant preparations that positively influence the morphological and biochemical blood composition, growth and productivity of poultry.

However, data on an effect of feed additives with lithium on poultry meat quality are absent in scientific literature.

In this connection, the aim of the research was to study an effect of the feed additives Peak anti-stress and SPAO

(SPAO-complex) — a stress protector and antioxidant for animals, which were developed by us, on broiler meat productivity and meat quality.

Materials and methods

The experiments were carried out in the Department of Food Engineering of the Ural State University of Economics and the Department of Morphology, Physiology and Pharmacology of the South Ural State Agrarian University.

The production experiments, testing and implementation were carried out on the chickens of the final hybrid of Arbor Acres cross in the conditions of AO «PRODO Tyumensky Broiler» in the Tyumen Oblast.

The chickens for the experiment were obtained from the breeding farm of the second order from the hens of the parent flock at the age of 245 days, the duration of egg storage was 5 days, the level of hatching was 88.9%. The chickens were kept in the poultry house with the usable space of 1288 square meters; density upon placing was 18.6 birds/m^2. The chicken body weight upon placing was 38.67 g; the floor temperature upon placing was 32.6–32.8 °C. The study was carried out in the conditions of the experimental facility with floor housing of broiler chickens in separate sections that spatially belonged to the same shop.

Three groups of broiler chickens with 6000 birds in each were selected for the experiment. The broiler chickens in the control group did not receive any pharmacological preparations and feed additives in addition to the main diet.

SPAO-complex was used in the second experimental group at a dose of 925 mg/kg liveweight; the scheme included five times of administration before slaughter. SPAO-complex was administered through the system of medicators Dosatron D25 RE0.2–2.0%. The content of lithium citrate was 10 mass%. In the third experimental group, the recipe composition for production of complete feed included the feed additive Peak anti-stress, which contained lithium carbonate (16.5–16.7 mass%) at a dose of 1693 g of the feed additive per 1 ton of feed or 440–552 mg per 1kg of chicken body weight. The proposed scheme included five times of feed additive administration daily during five days before slaughter.

The necessity to use the SPAO pharmacological complex during five days before slaughter of birds was substantiated by the scientific research carried out earlier by Miftakhytdinov A. V. et al. [20], who used different duration and doses of SPAO-complex administration to the hens of the parent flock during planed technological stresses. The period of five days facilitates the maximum effect and at the same time does not lead to reduction of the psychomotor reactions and appetite in birds and is linked with the pharmacokinetic characteristics of lithium salts.

The feed additive SPAO-complex was given to birds with water, as administration of pharmacological agents through the system of medicators (auto-dosing systems for pharmaceutical products) is the most convenient and, frequently, effective form of using pharmacological agents in the industrial poultry production. Medicators ensure a high degree of dose uniformity. SPAO-complex is a water soluble pharmacological agent and is used with water. Peak anti-stress is a feed additive with lower cost compared to SPAO-complex, contains water insoluble lithium carbonate and, therefore, was used in the composition of complete feed.

The broiler chickens of the control and experimental groups were fed according to the approved diets. The zootechnical analysis of complete feed showed the correspondence to the main regulated requirements. Feed moisture was on average 10.5% (with the norm of not more than 14.0% according to GOST 18221–99 «Mixed full-ration feeds for poultry. Specifications»), a level of metabolizable energy was 1.44 MJ (with the norm of not less than 1.34) the quantity of crude protein was within a normal range (19.0%). Complete feed had the optimal ratio of calcium and phosphorus of 1.9:1 and was also balanced by the main essential and non-essential amino acids, and had the optimal profile regarding lysine (100%): methionine + cystine — 77%, threonine — 69%, tryptophan 10%. Thus, the nutritional level of the diets for broiler chickens corresponded to the recommended requirements for raising Arbor Acres broilers.

The water supply was the same in the control and experimental groups. The zoohygienic conditions of keeping of the control and experimental groups were regulated with adherence to the technological regulations according to the requirements for the cross. The scheme of prophylaxis of viral and bacterial diseases corresponded to the approved veterinary regulations and instructions.

The chickens were slaughtered on the 38th day after hatching using the equipment of the «STORK» company (the line speed was 9000 birds/hour).

After slaughter, fifty birds from the first and second groups were examined for the following indices: the number of carcasses of the 1st category, number of carcasses sent to industrial processing, analysis of defects.

The indices of broiler meat productivity and meat quality were determined by conventional methods. Statistical analysis of the experimental data was carried out using the software package STATISTICA 12.

Results and discussion

To compare an effect of the feed additives on the growth and development of broiler chickens, the average daily gain, efficiency coefficient and broiler liveability were assessed. The obtained data suggest stimulation of the metabolic processes in the body of the birds due to reduction of pre-slaughter stress (Table 1).

By assessing the indices of the bird growth, the data were obtained that indicated an advantage of using feed additives.

During the experiment, the average daily gain was on average higher by 4.3% in the third experimental group compared to the control; this index increased by 1.8% compared to the second experimental group. This is explained by better intake of feed, which consumption increased to 22.5% on the six week of the experiment with feed conversion of 4.4%. Generally, the efficiency coefficient in the experimental

Table 1. Efficiency of using feed additives (n=50)

	Groups		
	1 group (control)	2 group (experimental)	3 group (experimental)
Average daily gain, g	57.30 ± 0.35	58.30 ± 0.41	59.77 ± 0.38
		P1 ≤ 0.0323	P_2 ≤ 0.0374
		P_3 =≤ 0.0224	
Liveability, %	94.42 ± 0.42	95.01 ± 0.51	96.57 ± 0.34
		P1 ≤ 0.1304	P_2 ≤ 0.0302
		P_3 ≤ 0.0388	
Feed conversion, feed/units	1.61	1.58	1.56
Efficiency coefficient	341.67	357.90	377.26

Note: P_1 — significance compared to the samples from the 1st group; P_2 — significance compared to the samples from the 2nd group; P_3 — significance when comparing the 1st group and the 2nd group

group was higher than in the control by 35.6 units, which was quite significant compared to the prototype (16.2 units). Broiler liveability in the third experimental group increased to 96.6%, which was 2.2% higher than in the control; in the second group, broiler liveability was 0.6% higher than in the control.

The final period of chicken fattening is characterized by the highest waste of population. It is during this time that stresses linked with the high intensity of the metabolic processes (productivity reaches 80–100g/day/chicken during this period) and high density of birds (due to their large weight in this period) facilitate a decrease in immune system effectiveness and lead to high mortality linked with activation of viruses and bacteria. The use of anti-stress and antioxidant agents during final fattening and vaccination of the parent flock of hens [20,21] facilitate maintenance of normal functioning of the immune system and prevention of the development of social and heat stress, which finally influences overall broiler liveability. Feed conversion is calculated in arbitrary units; in absolute terms, conversion increased only by 0.03 g in the second group and by 0.05 g in the third group, which is quite possible when using anti-stress agents and was vividly demonstrated in the performed investigations.

Table 2 presents meat productivity of broiler chickens. Meat yield was higher by 0.6% in the second experimental group and by 1.3% in the third experimental group compared to the control. In the second experimental group, the level of yield of carcasses of the first category increased up to 56.2% and technical waste reduced by 0.4%. The high yield of the bird carcasses of the first category (up to 79.1%) indicates an advantage of the method of using. The chicken carcasses of the third group differed from the first control group and the second experimental group by the number of defects. Technological defects associated with primary poultry processing (scalding, plucking, evisceration and chilling) were not taken into account, as the preparation and the feed additive did not influence their presence or absence. The number of black-and-blue marks, bruises and hematomas reduced by 23.5%, sprains and closed fractures by 20.0%, open fractures by 33.3% in the third experimental group compared to the control. In the second experimental group, the number of black-and-blue marks, bruises and hematomas reduced by 29.4%, sprains and closed fractures by 60.0%, open fractures by 33.3%. The obtained data indicate a decrease in the pre-slaughter injuries when using the feed additives and an advantage of Peak anti-stress.

According to the results of the organoleptic assessment of broiler chickens after slaughter, the control and experimental samples corresponded to the requirements of GOST 31962–2013 «Chicken meat (carcasses of chickens, broiler-chickens and their parts). Specifications» for the category «fresh meat»:

— the surface of the carcasses had the whitish-yellow color with the pinkish tint;

— subcutaneous and internal fatty tissue had the pale yellow color;

— the serous membrane of the thoracoabdominal cavity was moist, shiny, without slime and molds;

Table 2. Meat productivity of broiler chickens (n=50)

	Group		
	1 group (control)	2 group (experimental)	3 group (experimental)
Slaughter yield of eviscerated carcass, % (to liveweight)	72.8	73.4	74.1
Including the first grade, %	42.7	56.2	79.1
the second grade, %	57.3	43.8	20.9
Technical waste, %	15.6	15.5	15.2
Quantity of injuries received during poultry slaughter:			
Including black-and-blue marks, bruises and ematomas, units	34	26	24
sprains and closed fractures, units	5	4	2
open fractures, units	3	2	2

— muscles were slightly moist on the cut surface, did not leave a moist spot on the filter paper, were firm and elastic, the pit formed when pressing by the finger quickly straightened;

— odor was specific, typical for fresh poultry meat.

Table 3 presents the data on the comparative analysis of the tasting results of cooked meat and broth from birds of the control and experimental groups using the 9-point scale.

As follows from the data in Table 3, all samples of meat and meat broth from broiler chickens of the experimental and control groups had high indices with higher scores in the experimental samples. In terms of appearance, meat of the experimental broiler groups did not differ from the control and received the same average score — 8.11 points. Appearance and color on the cut surface were characterized as follows: «very beautiful», «beautiful», «good». When assessing taste of meat and meat broth from broiler chickens of the experimental and control groups, the following characteristics were used: «very tasty», «tasty», «quite tasty». The differences in the scores between the second and third experimental groups by appearance and color on the cut surface and taste were 0.11 and 0.10 points; they were 0.22 and 0.10 points by appearance of meat broth compared to the control samples. The differences in the meat sensory indices (odor, juiciness and consistency) were more pronounced. According to the results of tasting of meat broth, the experimental groups had higher average scores by 0.21 and 0.13 points, respectively. The characteristics «good», «beautiful», «very beautiful» were given to this indicator. Odor of meat and meat broth from broiler chickens was described with the attributes «quite aromatic», «aromatic» and «very aromatic». The number of points in meat assessment were higher in the second and third experimental groups by 0.22 and 0.18 points, in assessment of meat broth by 0.12 and 0.04 points, respectively. The experts characterized meat juiciness as «very juicy», «juicy» and «quite juicy» with the prevalence of the first two characteristics in the experimental groups, which influenced the average score being higher by 0.50 and 0.41 points. Consistency of broiler meat in the experimental groups was higher by 0.22 and 0.18 points and was mainly characterized as «very tender» and «tender».

In general, the scoring of meat and meat broth from broiler chickens of the experimental groups showed the superiority of their sensory indices over the results of the control group by 0.17–0.20 points and 0.13–0.21 points, respectively, which corresponded to the level of very high quality.

Therefore, changes in the sensory indices of cooked meat and meat broth from broiler chickens of the experimental groups indicated an increase in the consumer properties of meat products.

Assessment of the chemical composition of poultry meat demonstrated pronounced changes in the protein and fat content. For example, the protein content was higher by 12.4 and 15.2% in white meat, by 1.2 and 4.1% in red meat and by 18.8 and 5.8% in minced meat from birds of the second and third groups, respectively, compared to the control. With that, in the group, in which the feed additive Peak anti-stress was used, the protein content in fillet, legs and minced meat was 2.5, 2.9 and 2.0% higher, respectively, compared to the group of birds, which received SPAO-complex. It should be noted that the use of feed additives allowed bringing the protein content in white meat to the regulatory levels, which conditioned the strict correspondence of produced meat to the 1st category of quality and the higher biological and nutritional value (Table 4).

Somewhat different picture was observed for the fat content in meat. This parameter in white meat from birds of the experimental groups was 37.7% and 27.9% higher than in the control, respectively. However, the fat level in breasts of birds from the third experimental group was 7.1% lower compared to the second group. The fat content in red meat from the third experimental group was lower by 15.2% compared to the control and by 0.3% compared to the samples of meat from the second group. Similar results were obtained

Table 3. Results of tasting of cooked broiler meat and meat broth

	1 group (control)	2 group (experimental)	3 group (experimental)
Cooked meat			
Appearance	8.11 ± 0.93	8.11 ± 0.78	8.11 ± 0.67
Appearance and color on the cut surface	8.11 ± 0.78	8.22 ± 0.83	8.21 ± 0.75
Odor	8.11 ± 0.08	8.33 ± 0.07*	8.29 ± 0.12*
Taste	7.89 ± 0.78	8.07 ± 0.87	8.03 ± 0.47
Consistency (tenderness)	7.89 ± 0.07	8.11 ± 0.09*	8.07 ± 0.32
Juiciness	8.06 ± 0.08	8.56 ± 0.03*	8.47 ± 0.19*
Overall mean score	8.02 ± 0.31	8.22 ± 0.27	8.19 ± 0.36
Meat broth			
Appearance	8.22 ± 0.67	8.44 ± 0.73	8.32 ± 0.67
Aroma	8.21 ± 0.83	8.33 ± 0.71	8.25 ± 0.83
Taste	8.22 ± 0.67	8.44 ± 0.73	8.36 ± 0.67
Overall mean score	8.20 ± 0.47	8.41 ± 0.36	8.33 ± 0.47

Note: * — is significant at p ≤ 0.05 relative to the control group

Table 4. Chemical composition of broiler meat

	group		
	1 group (control)	2 group (experimental)	3 group (experimental)
White meat (fillet)			
Mass fraction of protein, %	20.52 ± 0.81	23.06 ± 0.47	23.64 ± 0.66
		$P_1 \leq 0.0412$	$P_2 \leq 0.0454$
		$P_3 \leq 0.3745$	
Mass fraction of fat, %	1.54 ± 0.21	2.12 ± 0.29	1.97 ± 0.13
		$P_1 \leq 0.0478$	$P_2 \leq 0.0423$
		$P_3 \leq 0.321$	
Red meat (leg)			
Mass fraction of protein, %	17.50 ± 0.07	17.72 ± 0.13	18.23 ± 0.18
		$P_1 \leq 0.0381$	$P_2 \leq 0.0352$
		$P_3 \leq 0.0322$	
Mass fraction of fat, %	8.53 ± 0.73	7.25 ± 0.56	7.23 ± 0.69
		$P_1 \leq 0.3457$	$P_2 \leq 0.4753$
		$P_3 \leq 0.5278$	
Minced meat			
Mass fraction of protein, %	18.81 ± 0.75	19.91 ± 0.76	20.31 ± 0.97
		$P_1 \leq 0.2352$	$P_2 \leq 0.3561$
		$P_3 \leq 0.3347$	
Mass fraction of fat, %	9.71 ± 0.66	8.83 ± 0.54	8.51 ± 0.75
		$P_1 \leq 0.2453$	$P_2 \leq 0.2143$
		$P_3 \leq 0.2344$	

Note: P_1 — significance compared to the samples from the 1st group; P_2 — significance compared to the samples from the 2nd group; P_3 — significance when comparing the 1st group and the 2nd group

regarding the fat content in minced meat; however, in this case, the difference between the third and second experimental groups was 3.6%. According to the reference data, the recommended protein content in red meat is not less than 18.0%. In our study, only the use of the feed additive Peak anti-stress allowed achieving this regulatory level.

Generally, the obtained results are indicative of the higher protein content in poultry meat of the experimental groups and reduction of red meat fattiness. With that, the most pronounced changes were noticed in the group of birds that received the feed additive Peak anti-stress.

Functional-technological properties of minced meat (water binding capacity (WBC), water holding capacity (WHC), emulsifying capacity (EC) and stability of emulsion (SE)) made from meat of the control and experimental groups were studied (Figure 1).

It follows from Figure 1 that the minced meat samples from the experimental groups were distinguished by higher functional-technological properties. For example, WBC was higher by 3.8% and 4.5%, WHC by 4.4% and 5.9%, EC by 2.0% and 2.3%, SE by 2.2% and 3.1%, respectively, in the samples of the second and third experimental groups compared to the control.

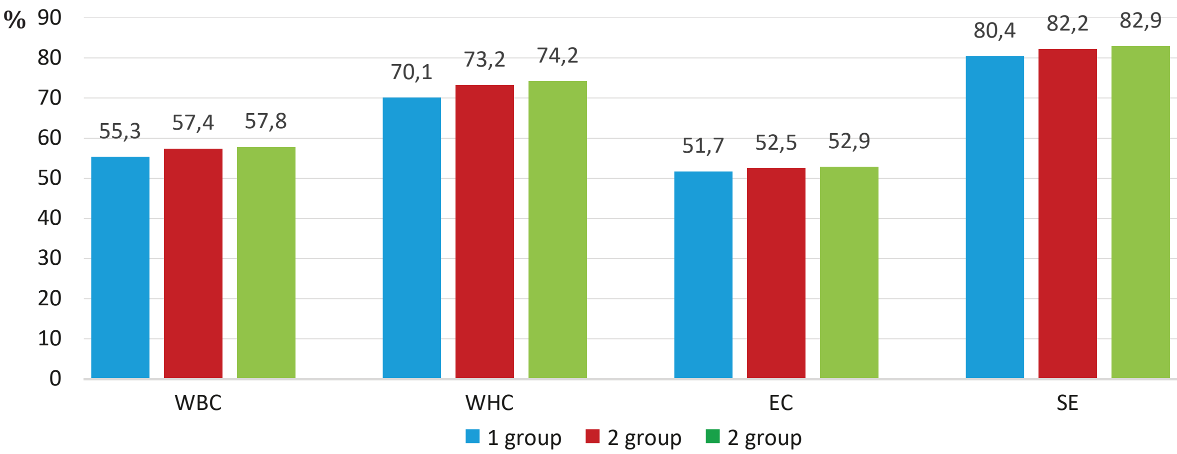

Figure 1. Functional-technological properties of minced meat (water binding capacity (WBC), water holding capacity (WHC), emulsifying capacity (EC) and stability of emulsion (SE)) made from meat of the control and experimental groups

Taking into consideration the production indices, results of slaughter and costs for broiler chicken production, economic efficiency and profitability of feed additive using were assessed.

According to the conventional methodology used in veterinary medicine (1997) and recommendations of Zhuravel N. A. [22], economic efficiency of veterinary measures per a ruble of costs expresses the relation of an economic effect (a positive economic result) to total costs on these veterinary measures (including a cost of a preparation and labor costs of its using). In our experiment, with regard to the obtained additional profit due to an increase in meat productivity and meat quality in the second experimental group, where SPAO-complex was used, economic efficiency per one ruble of costs was 0.19 rubles, in the third experimental group it was 8.67 rubles, which clearly indicates an advantage of using the feed additive Peak anti-stress in economic terms.

In the process of activation of glucogenolysis and glycolysis, the release of catecholamines, glucagon and corticosterone into the blood occurs. The catabolic effect of corticosterone is manifested in breakdown of amino acids to provide for energy expenditure of the body and mobilization of fatty acids; it is this fact that is linked with a decrease in meat productivity and poultry meat quality.

Therefore, the use of feed additives allows activation of the adaptation processes in the body of birds and stabilize metabolism due to the combined stress protective and antioxidant action, which is evident from an increase in the average daily gain of broiler chickens on the background of using SPAO-complex and Peak anti-stress by 1.8–4.3%, meat yield by 0.6–1.3%, yield of the carcasses of the 1st category of up to 56.2–79.1%, as well as from an improvement in the organoleptic properties of meat, an increase in the protein content in white and red meat and an increase in functional-technological properties of minced meat. The use of the feed additives is economically profitable.

Conclusion

To increase stress resistance of birds in the conditions of industrial production, one of the main factors is the use of a balanced diet enriched with a vitamin and mineral complex, probiotic additives and additives with the antioxidant properties. Studies proved the efficiency of feed additives with microelements, including lithium salts as a stress protector regarding meat productivity of poultry. The results of the study on the effect of the feed additives Peak anti-stress and SPAO-complex with different lithium content on broiler meat productivity and meat quality show an increase in the average daily gain by 1.8% and 4.3%, respectively, and an increase in the biological value of poultry meat. It is necessary to take into account the obtained results in the technological processes upon broiler production.

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Review paper

BIOLOGICALLY ACTIVE PEPTIDES OF MEAT AND MEAT PRODUCT PROTEINS: A REVIEW

PART 2. FUNCTIONALITY OF MEAT BIOACTIVE PEPTIDES

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Keywords: *biologically active peptides, meat proteins, the functionality, ACE-I, antioxidant activity, antithrombotic activity, antimicrobial activity, anticancer effect, opioid effect, bioavailability*

Abstract
Biologically active peptides (BAP) are regarded as the main products of protein hydrolysis. BAP activity depends on the amino acid sequence molecular weight and chain length, type and charge of an amino acid at the N-terminus and C-terminus, hydrophobic and hydrophilic properties, spatial structure. They positively influence many systems of the human body, including the blood circulatory, nervous, immune, gastrointestinal and other systems. The health-improving effect of bioactive peptides is formed due to their antioxidant, antihypertensive, antithrombotic, immunomodulatory, antimicrobial, anti-allergic, opioid, anti-inflammatory, hypocholesterolemic and anticancer properties. Angiotensin-I-converting enzyme (ACE) inhibitory peptides are most studied due to their effect on blood pressure regulation. Unlike synthetic preparations, biologically active peptides do not have side effects and, therefore, can be used as their alternative. There is a growing commercial interest in peptides generated from meat proteins is in the context of health saving functional foods. The paper describes prospects, pros and cons of using bioactive peptides as functional food ingredients and biologically active food additives.

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Introduction

Native amino acid sequences and peptides generated during meat autolysis or enzymolysis can be functionally active. Bioactive peptides (BAPs) can be generated during thermal or other technological processing. Bioactive peptides with the antioxidant, antithrombotic, antimicrobial, hypotensive, immunomodulatory, opioid and other biological effects, which have curative or preventive impact on pathogenesis of several diseases, have been discovered and studied [1,2,3,4].

A significant number of peptides with different activities have already been isolated from meat raw materials. These peptides contain 2 to 9 (to 25 sometimes) hydrophobic amino acid residues in a strict sequence [5]. Many of them were obtained by both *in vivo* and *in vitro* proteolysis with enzymes of different origin [6].

At present, the interaction between the activity and structure of peptides has been studied. The peptide activity depends on the amino acid sequence, molecular weight and chain length, type and charge of an amino acid at the N-terminus and C-terminus, hydrophobic and hydrophilic properties, spatial structure, etc. Peptides with higher ACE inhibitory activity usually have aromatic or basic amino acids at the N-terminus, more hydrophobic and positively charged amino acids at the C-terminus [7].

Many natural BAPs structurally differ from those formed as a result of the protein post-translational modification. They contains non-protein amino acids (β-alanin, γ-aminobutyric acid), D-amino acids, alkylated amino acids. The H-peptide bonds and cyclic structures are the characteristic of low molecular weight peptides. Along with residues of pyroglutamic acid, they provide protection from proteases with substrate specificity to peptides from α-amino acids with normal bonds, which allows retention of peptide functionality up to the moment of their absorption [1,8].

Main part

1. Angiotensin-I-converting enzyme (ACE) inhibitory peptides

Angiotensin-I-converting enzyme (ACE) inhibitory peptides are the most studied meat BAPs, probably due to their effect on blood pressure regulation. ACE-I is an enzyme dipeptidyl carboxypeptidase, which converts angiotensin-I (decapeptide) into angiotensin-II (octapeptide), which leads to constriction of arteries and, consequently, to an increase in blood pressure. In this connection, ACE inhibition can be linked with prophylaxis of cardiovascular diseases [9,10].

ACE-I inhibitory peptides are small peptides with 2 to 20 amino acids. Their function depends on a protein, conditions and a degree of hydrolysis, molecular weight and amino acid composition, as well as a position of amino acids in peptide sequences. ACE-I inhibitory peptides have hydrophobic amino acids and branched-chain amino acids in their structure [11,12].

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Katayama et al. found two ACE-I peptides (KRQKYD, EKERERQ), which showed the hypotensive activity on rats. They were obtained by hydrolysis of pork with pepsin [13].

The other peptide with similar function, RMLGQTPTK (44–52 position of troponin C) was also isolated by treatment of a porcine skeletal muscle with pepsin [14].

Using pepsin and pancreatin, 22 ACE-I peptides were isolated; peptide sequences KAPVA and PTPVP showed the highest antihypertensive effect [15].

Peptides with ACE inhibitory activity were found in the hydrolysate of a porcine skeletal muscle as a result of the complex digestion by eight enzymes — proteases. Peptides MNPPK and ITTNP showed the highest activity [16].

Peptides isolated from connective tissue were also identified as ACE inhibitors. Bioactivity of collagen peptides depend on the quantity of glycine and proline [17].

Properties with ACE-I effect were found *in vitro* and *in vivo* in peptides AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP and PAGNPGADGQPGAKGANGAP isolated from a hydrolysate of bovine Achilles tendon collagen using bacterial collagenase. Both peptides showed the ACE-I activity in rats [18].

Peptides from beef tendons (GPRGF, SPLPPPE, EGPQGPPGPVG and PGLIGARGPPGP), which showed high ACE- and renin-inhibitory activities, were also identified [19,20].

Peptides with the *in vivo* ACE-I activity were isolated from chicken collagen treated with protease from *Aspergillus oryzae* [21].

Peptides from protein hydrolysate of a mixture of chicken combs and wattles were obtained by enzymatic hydrolysis. The protein hydrolysate showed the anticoagulant capacity and high ACE-inhibitory activity. The peptides were identified by LC–MS sequencing. From the pool of sequenced peptides, three peptides were selected and synthesized based on their low molecular weight and the presence of amino acids with ACE-inhibitory potential at the C-terminus: peptide I (APGLPGPR), peptide II (Piro-GPPGPT), and peptide III (FPGPPGP). Peptide III demonstrated the highest ACE-inhibitory capacity [22].

ACE-inhibitory peptides were found in hemoglobin. For example, peptides LGFPTTKTYFPHF and VVYPWT, which corresponded to the 34–46 fragment of the α-chain and the 34–39 fragment of the β-chain of porcine hemoglobin, were obtained by hydrolysis of pork blood with pepsin [23].

2. Peptides with antioxidant activity

It is known that antioxidants are beneficial for human health as they can protect the body from molecules known as reactive oxygen species (ROS), which can attack membrane lipids, proteins and DNA. Antioxidant peptides were found in many food products including milk, wheat, potato and mushrooms. The antioxidant activity of peptides can be determined by their ability to reduce stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH assay) [24].

Over the last several years, many studies have been focused on antioxidant peptides obtained from fish, while studies on antioxidant peptides from hydrolysates of farm animal's muscles are limited [25].

Two peptides that inhibited linoleic acid autoxidation in the model system were identified in the extract of chicken essence extract. The first peptide HVTEE with the induction period of 2.49 days, the second PVPVEGV had the induction period of 6.5 days [26].

The hydrolysate with the potent antioxidant activity was obtained after cleavage of venison with papain. Two peptides that inactivate hydroxyl, DPPH, superoxide, and peroxyl radicals were identified: APVPH I (MQIFVKTLTG) and APVPH II (DLSDGEQGVL) [27].

Several peptides isolated from meat have the antioxidant activity due to their ability to inhibit lipid peroxidation, chelate metal ions, inactivate free radicals and reactive oxygen species [28,29].

The most important antioxidants natively existing in meat are dipeptides carnosine and anserine, which show their antioxidant activity chelating pro-oxidative metals [30].

Also, peptides with the antioxidant activity can be generated by hydrolysis of meat raw materials with certain proteases.

Saiga et al. [30] in studies *in vitro* of porcine myofibrillar proteins hydrolyzed with papain and actinase E, found five peptides (DSGVT, IEAEGE, EELDNALN, VPSIDDQEELM and DAQEKLE), which showed antioxidant activity using the in the linolenic acid peroxidation system.

Three peptides (ALTA, SLTA and VT) obtained from actomyosin of porcine skeletal muscles actomyosin showed the antioxidant activity in rats not only *in vitro* but also *in vivo* in rats [31].

Li et al. [32] isolated four antioxidant peptides (QGAR, LQGM, LQGMH and LC) from pork collagen using animal, plant and microbial enzymes (pepsin, papain, protease from bovine pancreas and bacterial proteases from *Streptomyces* spp. and *Bacillus polymyxa*) in different combinations.

Banerjee and Shanthi [18] isolated a peptide from bovine tendon collagen that consisted of contains 36 amino acid residues. Peptide was able to inactivate free radicals and chelate metals, from bovine tendon collagen.

Peptides with the antioxidant activity can be found in finished meat products. For example, 27 antioxidant peptides from Spanish dry-cured ham were sequenced using LC–MS/MS [33]. The highest activity was revealed in peptides SAGNPN and GLAGA.

Broncano et al. [34] isolated two peptides FGG and DM with the antioxidant activity from pork «chorizo» sausages.

Xing et al. [35] described several antioxidant peptides from dry-cured Xuanwei ham. Peptide DLEE was with the highest antioxidant activity.

3. Peptides with antithrombotic properties

Peptides that inhibit aggregation of blood platelets are recommended as diet components facilitating prophylaxis of thrombosis, which often occurs in patients with ischemic heart disease or other diseases of the blood circulatory system. One of the stages of platelet aggregation is non-covalent binding of the α- and β-fibrin chains (fibrin polymerization). The N-terminal fragment with the sequence GRP of the α-chain is the key factor of fibrin polymerization [36].

Luzak et al. [37] found that DGEA (Asp-Gly-Glu-Ala) sequence is type I collagen recognition motif, which significantly inhibited blood platelets adhesion, aggregation and release reaction by collagen.

Morimatsu et al. [38] and Shimizu et al. [39] isolated peptides that showed the antithrombotic activity from porcine muscle *longissimus dorsi* hydrolyzed with papain. In particular, Shimizu et al. [39] examined antithrombotic activity both *in vitro* by the platelet function test using rat blood and *in vivo* by oral administration to mice at a dose of 70 mg/kg body weight. The *in vivo* results showed that meat the peptide significantly reduced carotid artery thrombosis and the platelet activity and the effect is comparable to aspirin treatment at a dose of 50 mg/kg body weight.

4. Hypocholesterolemic peptide

Increased cholesterol concentrations in plasma are linked with an increased risk of cardiovascular diseases. Cholesterol enhances lipid peroxidation, protein oxidation and generation of free radicals (H_2O_2), disrupts the antioxidant system (SOD, CAT, GPx and GSH), as well as the activity of ATPase and causes histopathological disorders. Bioactive peptides can be regarded as an alternative for prophylaxis or treatment of these disorders. Peptides identified as hypocholesterolemic include lactostatin (IIAEK), enterostatin (VPDPR), peptides DPR, LPYP and LPLPR [40]. It is assumed that peptides of this type have different mechanisms of action and can increase excretion of bile acids with feces, bind with phospholipids and exert the activity of the cholesterol level reduction in human serum.

Nagaoka et al. hydrolyzed bovine β-lactoglobuline with porcine trypsin to obtain peptide IIAEK [41]. This peptide had the highest hypocholesterolemic effect in rats compared to β-sitosterol, a plant microelement with the chemical nature similar to the cholesterol nature and well known for its therapeutic potential, for example, the antioxidant, antidiabetic, anticancer, antihyperlipidemic, immunomodulatory and other effects [42].

When studying the protein profile of raw smoked sausages produced with starter cultures, including those that were capable to reduce the cholesterol level in the culture medium and finished meat products, a change in protein fractions and presumptive formation of BAPs were shown. A wide range of peaks of peptide masses that had certain differences was obtained by MS. Analysis of rat blood serum lipids revealed the highest decrease in the cholesterol concentration in the group with starter culture the composition from the collection of MGUPP (*Lactobacillus sakei* 104, *Pediococcus pentosaceus* 28 and *Staphylococcus carnosus* 108) compared to the control, mainly, due to a 3 fold decrease in LDL cholesterol (P<0.05) [43,44,45].

5. Peptides with antitumor activity

It is known that several peptides can also show the antitumor activity, inhibit cell proliferation and exert the cytotoxic action on cancer cells [46]. Jang et al. [47] studied the activity of four peptides isolated from beef sarcoplasmic proteins regarding breast, stomach and lung adenocarcinoma cells. The results showed that peptide GFHI had the highest cytotoxic effect on breast cancer cells and reduced viability of stomach adenocarcinoma cells. Peptide GLSDGEWQ showed the inhibitory effect on proliferation of stomach adenocarcinoma cells.

Yu et al. antitumor effect analyzed bioactive peptide-3 (ACBP-3) isolated from goat liver antitumor effect on gastric cancer stem cells (GCSCs) *in vitro* and *in vivo*. ACBP-3 decreased the percentage of CD44(+) cells, suppressed the proliferation of the SC (spheroid colonies) cells and inhibited their clone-forming capacity in a dose dependent manner. Tumor formation from GCSCs occurred substantially longer when the cells were treated with ACBP-3 *in vivo* [48].

6. Peptides with antimicrobial activity

Biologically active peptides with the antimicrobial properties were identified in microorganisms, animals and plants. Antimicrobial peptides of animal origin show the inhibitory activity towards much larger spectrum of microorganisms than those produced by bacteria, while the latter demonstrate higher effectiveness at extremely low concentrations even of the nano level. However, the antimicrobial peptides have certain common properties. A majority of antimicrobial peptides have 50 amino acids and less; about 50% of them are hydrophobic amino acids and often make amphipathic 3-D structures [49,50].

Several peptides with the antimicrobial activity were isolated from beef blood; however, only one study showed the presence of antimicrobial peptides obtained from meat [47]. In this study, Jang et al. [47] isolated four peptides (GLSDGEWQ, GFHI, DFHING and FHG) after hydrolysis of beef sarcoplasmic proteins with commercial enzymes. All peptides were then tested on the antimicrobial activity against six pathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes*). The results showed different antimicrobial effect on one or several bacteria. All peptides were active against *P. aeruginosa*. Peptide GLSDGEWQ demonstrated the inhibitory action on *E. coli*, *S. typhimurium*, *B. cereus* and *L. monocytogenes*.

Peptides of the hemoglobin α-chain show the antimicrobial activity after treatment with pepsin. Adje et al. [51] described such action of peptides TKAVEHLDDLPGALSELSDLHAHKLRVDPVNFKLLSHSLL and LDDLPGALSELSDLHAHKLRVDPVNFKLLSHSL of the 67–106 fragment of the hemoglobin α-chain regarding *Kocuria luteus*, *Listeria* *innocua*, *Escherichia coli* and *Staphylococcus aureus*. Daoud et al. described the action of peptide VTLASHLPSDFT-PAVHASLDKFLANVSTVL obtained in the similar way against *Micrococcus luteus* A270, *Listeria innocua*, *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus saprophyticus* and *Staphylococcus simulans* [52].

Catiau et al. determined a minimal number of amino acid residues in a peptide that showed the antimicrobial properties and was isolated from the hemoglobin α-chain. It was tripeptide KYR. The experiments demonstrated the inhibitory activity both against gram-negative microorganisms *Escherichia coli*, *Salmonella enteritidis*, and gram-positive ones — *Listeria innocua*, *Micrococcus luteus*, *Staphylococcus aureus* [53].

7. Opioid peptides

Opioid peptides received their name due to their ability to interact with opioid receptors and impact on the nervous system of the body [54]. They can reduce pain similar to opiates and affect an emotional condition. Opioid peptides are endogenous ligands of opiate receptors. There are three main classes of endogenous peptides. Enkephalins are neuropeptides with the morphine-like effect. Leucine-enkephalin H_2N-Tyr-Gly-Gly-Phe-Leu-COOH (with the molecular weight of 556) and methionine-enkephalin H_2N-Tyr-Gly-Gly-Phe-Met-COOH (with the molecular weight of 574) were discovered in 1975. Dynorphins are peptides that contain a high proportion (almost one third) of basic amino acid residues, in particular lysine and arginine. Dynorphins have up to 41% of hydrophobic residues [55]. The main action of dynorphins is associated with the activation of κ-opioid receptors. The dynorphin analgesic effect is six times higher than that of morphine [56]. Endorphins block transmission of pain impulses. The structural formula of endorphin is NH_2-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln-COOH. Opioid peptides are involved in regulation of the hormonal activity and influence on the immune system. An effect of opioid peptides on brain function regulation was established [57]. Animal proteins can become a source of opioid peptides [58].

A method for quantitative assessment of opioid peptides dynorphin A1–8 and [Met5]-enkephalin-Arg6-Gly7-Leu8 (MERGL) was described. The quantity of prodynorphin and dynorphin A1–8 increased with the beginning of the inflammatory process in the spinal cord and did not reduce during the inflammation resulted from administration as the inflammatory agent, carrageenan. The quantity of the enkephalin precursor also increased, more as a result of nerve injury rather than as a response to inflammation [59].

An opioid effect of fragments 143AYFYPEL149 and 144YFYPEL149 of alpha S1 casein, which stimulated gene MUC5A expression, was revealed in the samples of ileum in the experiments on guinea pigs [60].

It was found that several food-derived peptides influence rodent behavior. These peptides can be formed in the process of food digestion and can affect opioid receptors and cross the gastrointestinal and blood-brain barriers without their injury. An increased consumption of peptides from wheat gluten (gliadorphin-7) and milk casein (β-casomorphins) can be a factor linked to the development or maintenance of schizophrenia symptoms. Also, peptides derived from spinach (rubiscolins) and soy (soymorphins) influenced spontaneous behavior and memory [61].

8. Bioavailability of bioactive peptides

For bioavailability assessment it is necessary to determine whether the BAP can reach its target site in an active form and sufficient amount to affect health. The action of gastrointestinal enzymes such as intestinal absorption, cellular uptake and action of blood plasma peptidases, can modify the peptide structure or hydrolyze them leading to losses, retention or an increase of bioavailability [62,63,64].

Small peptides are more resistant to destruction by intestinal enzymes and more easily absorbed [65]. Ohara et al. [66] found small peptides obtained from collagen in blood after oral administration of protein hydrolysate products.

On the other hand, cell models, such as heterogeneous monolayers of human epithelial colorectal adenocarcinoma cells (Caco-2 cells) are effective to study transepithelial transport machanisms of peptides [67,68,69].

Shimizu et al. [70] reported that chicken collagen octapeptide GAXGLXGP can be transported through the human intestinal epithelium. Fu et al. [20] identified two peptides (VGPV and GPRGF) obtained from bovine collage and showed their ACE-I inhibitory activity on human intestinal epithelial Caco-2 cells highlighting bioavailability of these peptides.

An ability of peptides to withstand enzymatic splitting and be transported through intestinal cell membranes into the bloodstream depends on their characteristics, length and amino acid composition. Proline-rich peptides are more resistant to gastrointestinal enzymes. Di- and tripeptides can be absorbed intact by the peptide transport systems and hydrolyzed later [65]. The low transport ability of oligopeptides compared to di- and tripeptides is probably because of their length and may involves the paracellular route, while the hydrophobicity of peptides apparently does not influence the absorption [71].

In addition, the absorption of peptides can be influenced by co-existing peptides and food components, which can share the transport pathway or take part it its regulation [71].

Bioavailability of BAP can also be influenced by the conditions of their processing/storage and food matrix — peptide interactions, which can lead to the modification of peptides with alterations in their native structure and activity [72].

9. Challenges and limitations in the field of BAP research

As the basic principles of bioactive peptide detection are known today, it is necessary to continue research of bioactive peptides to a better understanding of their effects, interactions and bioavailability. Several authors argue that it is necessary to consider seriously interactions of a food matrix, especially when the aim is the use of bioactive peptides as a functional ingredient [72]. More and more often, when a peptide has been identified in a food matrix, it is synthesized and characterized as an individual molecule. However, the expected *in vitro* and/or *in vivo* activity can be different, when a peptide interacts with a complex mixture of compounds being a part of the composition of any product. On the other hand, more efforts towards the development of quantitative methodologies are needed to better understanding the processes of peptide hydrolysis, biological activities and/or bioavailability.

Data such as a number of specific naturally generated peptides in an initial food sample or a dose of a bioactive peptide that is necessary for an *in vivo* effect, as well as final sequences and the quantity in the bloodstream and target organ after gastrointestinal digestion are crucial for assessment of a BAP therapeutic and heeling effect on human health. Detection in a meat sample of the quantity of bioactive peptides that are able to reach a target site in the human body, has a fundamental importance in the studies of bioavailability for a better understanding of effects and mechanisms of action of these peptides. The main limitation for quantification is the nature of a sample: high complexity of a matrix and the fact that small peptides often consist of less than four amino acids at their low concentration in a sample. Modern achievements in mass spectrometry analysis, bioinformatics and updated protein databases facilitate progress in quantitative proteomics [72].

Bioactive peptides obtained from meat are promising candidates for functional ingredients of specialized or healthy foods due to their biological properties [73].

Preparations based on meat peptides with functional groups have not been commercialized by the industry; therefore, foods with functional meat peptides can open a new market of specialized products.

Conclusion

1. The interest to native peptides is to a great extent due to their unusually high biological activity. They exert the potent pharmacological action on many physiological functions of the human body. At the same time, their low stability and rapid decomposition in the body at the physiological pH values were noticed. All this contributed to the development of research on isolation and study of the functional effect of peptides isolated from animal organs and tissues.
2. At present, bioactive peptides with the antioxidant, antithrombotic, antimicrobial, hypotensive, immunomodulatory, opioid and other biologically effects, which have curative or preventive impact on pathogenesis of several diseases, have been discovered and studied. Both native amino acid sequences and peptides generated during meat autolysis or enzymolysis, thermal or other technological processing can be functionally active.
3. The peptide activity depends on the amino acid sequence, molecular weight and chain length, type and charge of an amino acid at the N-terminus and C-terminus, hydrophobic and hydrophilic properties, spatial structure.
4. Angiotensin-I-converting enzyme (ACE) inhibitory peptides are the most studied meat bioactive peptides due to their effect on blood pressure regulation.
5. Several peptides isolated from meat have the antioxidant activity due to their ability to inhibit peroxidation of lipids, chelate metal ions, inactivate free radicals and reactive oxygen species.
6. Peptides that inhibit aggregation of blood platelets are recommended as diet components facilitating prophylaxis of thrombosis, which often occurs in patients with ischemic heart disease or other diseases of the blood circulatory system.
7. Increased cholesterol concentrations in plasma are linked with an increased risk of cardiovascular diseases. Cholesterol enhances lipid peroxidation, protein oxidation and generation of free radicals (H_2O_2), disrupts the antioxidant system (SOD, CAT, GPx and GSH), as well as the activity of ATPase and causes histopathological disorders. Bioactive peptides can be regarded as an alternative for prophylaxis or treatment of these conditions.
8. Several bioactive peptides show the antitumor activity, inhibit cell proliferation and exert the cytotoxic action on cancer cells.
9. Antimicrobial peptides of animal origin show the inhibitory activity towards a large spectrum of microorganisms. Several peptides with the antimicrobial activity were isolated from beef blood.
10. Animal proteins can become a source of opioid peptides.
11. An ability of peptides to withstand enzymatic denaturation and be transported through intestinal cell membranes into the bloodstream depends on their characteristics, length and amino acid composition.
12. The expected *in vitro* and/or *in vivo* activity can be different, when a peptide interacts with a complex mixture of compounds being a part of a product composition. Therefore, it is necessary to study interactions in the food matrix, especially when the aim is the use of bioactive peptides as a functional ingredient.
13. Ingredients with functional groups meat peptides that were either formed during technological treatment or already presented in raw meat can open a new market of E-free health promoting foods.

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BIOCHEMICAL AND HISTOLOGICAL INDICATORS OF BLOOD AND *M. LONGISSIMUS DORSI* OF YOUNG BULLS OF KAZAKH WHITE-HEADED BREED OF DIFFERENT GENOTYPES BY THE CAPN1 AND GH GENES

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Keywords: *kazakh white-headed breed, gene of growth hormone, calpain gene, histological, chemical analysis of muscle tissue, fatty acid composition of blood*

Abstract
The use of molecular genetic markers for productivity is one of the modern approaches in breeding beef cattle. The article presents the results of the studies on the influence of genotypes by the calpain (CAPN1) and growth hormone (GH) genes on live weight, fatty acid composition of blood plasma, quantitative and qualitative indicators of meat of young cattle of Kazakh white-headed breed. It was established that animals of the homozygous geno-types CC–CAPN1 and VV-GH had a larger live weight and its average daily gain on the 240th and 365th days. Their superiority over the GG and LL genotypes was 13.8% ($P<0.05$) and 6.9%; 14.6% ($P<0.05$) and 6.9%, respectively. The carriers of the desirable alleles in the homozygous state had the sum of unsaturated fatty acids higher by 6.3% and 7.1%, respectively, than the young bulls, in which genotypes they were absent. The predominance of unsaturated fatty acids over saturated fatty acids also determined lower values of lipid me-tabolism direction index (0.92 and 1.00) in the animals with the desirable genotype. Muscle tissue of animals of the CC and VV genotypes was characterized by a higher level of protein, fat by 0.28–2.13 abs. per cent, energy value, and the quantity of muscle fibers with a smaller diameter per unit area compared to the GG and LL genotypes by on average 8.7% and 25.5% ($P<0.01$). Due to the greater number of interfiber fat inclusions, muscle tissue of the desirable genotypes received higher marbling scores. The obtained data testify to the prospects of replication of animals, which genotype has the desirable alleles for further improvement of traits of meat productivity of Kazakh white-headed breed.

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Introduction

One of the main tasks in selective breeding is selection and breeding of animals with targeted economic traits. This result can be achieved by using genetic markers for productive traits. The use of genetic markers in beef cattle selection in combination with the main zootechnic methods for animal breeding make it possible to significantly accelerate the process of creation of herds with genetically preset potential of high productivity [1,2,3].

The calpain gene (CAPN1) can be considered one of the candidate genes that are directly associated with meat tenderness and marbling. The mechanism of its action consists in the fact that the calpain system creates conditions for uniform distribution of intramuscular fat between fibers on the basis of calcium-dependent cysteine protease due to decomposition of the so-called Z-discs of skeletal muscles and weakening of bonds between muscle fibers, which ensures the so-called marbling and corresponding meat tenderness and juiciness [4,5].

The biological effect of the growth hormone gene (GH) consists in regula-tion and stimulation of many metabolic processes. The hormone (somatotropin) controlled by GH is produced by the anterior lobe of the hypophysis and plays a key role in carbohydrate and lipid metabolism [6,7,8].

It is known that lipids are very active in terms of metabolism; they act not only as a source of energy but also have more significant functions — structural and regulatory. Lipids take part in the metabolic processes mainly as free saturated and unsaturated fatty acids. It is also necessary to note that fatty acids not only play a key role in the vital activities of the body and formation of productivity, but several of them act as signal molecules involved in the process of expression of individual genes; that is, the development of fat in fatty tissue of ruminants is operated and modulated by certain genes [9].

Several studies by foreign and Russian scientists revealed that the C allele of the CAPN1 gene and the V allele of the GH gene are desirable for selection regarding an increase in quantitative and qualitative indicators of beef cattle productivity [10,11,12,13,14,15,16].

Another aspect in assessment of the action of certain genes with regard to farm animal productivity is a complex analysis of quantitative and qualitative indicators of final products. For beef cattle, these indicators are an amount of produced meat, its nutritional properties, energy value, quality and safety, consumer properties, which are also determined by chemical, biochemical and microstructural indicators [17,18].

In this connection, the study of the fatty acid composition of blood plasma, meat productivity of beef cattle of Kazakh white-headed breed of different genotypes by the calpain and growth hormone genes, including biochemical

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and microstructural analysis of muscle tissue is of scientific and practical interest.

The above mentioned was a substantiation of the aim of the present work: to study the biochemical and histological indicators of blood and *m. longissimus dorsi* of young bulls of Kazakh white-headed breed of different genotypes by the CAPN1 and GH genes.

Materials and methods

The studies were carried out in the laboratory of immunogenetics and DNA technologies, laboratory of veterinary medicine and laboratory of morphology and quality of products of the All-Russian Research Institute of Sheep and Goat Breeding (VNIIOK) — the branch of the Federal State Budgetary Scientific Institution North Caucasian Agrarian Center (North Caucasian FNAC). The object of the research were young bulls of Kazakh white-headed breed (n=93) of SPK Kolhoz Gigant (Blagodarensky district, Stavropol Krai).

DNA was extracted from blood samples using the DIAto^{mtm}DNAPrep kit (Izogen, Moscow) according to the protocol of the manufacturer. Genotyping by the CAPN1 gene was performed using an analyzer of nucleic acids (ANK-32) by real time PCR with the «CAPN1» kit (Sintol, Russia), by the GH gene using PCR-RFLP with primers (GH-F: 5'- gct-gct-cct-gag-cct-tcg –3' and GH-R: 5'- gcg-gcg-gca-ctt-cat-gac-cct-3') and restrictase Alu I (NPO «SibEnzyme»).

The fatty acid (FA) content in blood plasma was determined by the method of gas-liquid chromatography in a form of methyl esters on a gas chromatograph «Cristal 200» with a capillary column HP-FFAP 50 m, 0.32 mm, 0.5 pm (USA). Fatty acid methyl esters were obtained using the method by Morrison and Smith. FAs were identified using the standards of Sigma and Fluka. Quantitative determination of FAs was carried out with the use of Chromatec Analytic software [19]. The lipid saturation index — a ratio between the sum of saturated fatty acids and the sum of unsaturated fatty acids, was used as an integral indicator that also characterizes the direction of lipid metabolism.

Analysis of the level of total lipids, cholesterol and glucose in blood was carried out according to the methods given in «Methods of veterinary clinical laboratory diagnostics: reference book» [20].

Dynamics of live weight of young bulls of different genotypes was deter-mined by their weighing at birth, on the 240th and 365th days. An average daily gain was calculated by the difference in the values and period of measurement.

During control slaughter of three animals of each genotype at the age of 12 months, samples of *m. longissimus dorsi* were taken for chemical analysis and histological investigations. An amount of moisture was measured according to GOST 9793–2016 «Meat and meat products. Methods for determination of moisture content» [21]. A meat sample was minced in a homogenizer, thoroughly mixed, dried in a drying oven at a temperature of 103±2 °C until constant air-dry mass. The mass fraction of moisture was determined by the difference between the sample weight before and after drying and expressed in percent. An amount of fat was determined according to GOST 23042–2015 «Meat and meat products. Methods of fat determination» [22], by extraction with petroleum ether from a minced and dried sample in the Soxhlet apparatus with the following removal of the solvent and drying of fat until the constant weight. An amount of protein was determined according to GOST 25011–2017 «Meat and meat products. Protein determination methods» [23] using the Kjeldahl method by an amount of nitrogen formed in min-eralization of organic substances in a minced and dried sample and its conversion to protein using the coefficient of 6.25. Caloricity was determined by calculation using V. M. Aleksandrov's equation:

$$C = [D - (F + A)] \times 4.1 + (F \times 9.3),$$

where C is caloricity, kcal; D, A, F — an amount of dry matter, ash and fat, respectively.

For histological investigations, the samples were fixed in the 10% solution of neutral formalin and then densified by pouring into gelatin. Sections with a thickness of 7–8 μm were obtained on a freezing microtome. Structural components of muscle tissue were revealed by the methods of staining using Carazzi's hematoxylin with sudan III according to Ehrlich. The muscle bundle area, the quantity of muscle fibers in a bundle, a diameter of a muscle fiber and bundle, a ratio of muscle and connective tissues were determined according to GOST 19496–2013 «Meat and meat products. The method of histological investigation» [24].

The obtained data were subjected to biometrical processing using Microsoft Office and BIOSTAT. Based on the mean values and standard errors, significance of difference between the mean values was calculated using the Student test.

Results and discussion

As a result of the performed genotyping, it was established that the frequency of the CC, GG, CG genotypes of the CAPN1 gene was 0.06, 0.81 and 0.13, respectively; the frequency of the VV, LL, LV genotypes of the GH gene was 0.31, 0.51 and 0.18, respectively. The revealed polymorphism allowed dividing animals according to the established genotypes and studying the biochemical composition of blood plasma in the young bulls with medium indicators of productivity according to a corresponding genotype, as well as performing chemical analysis and histological investigations of *m. longissimus dorsi*.

It was established that the young bulls of Kazakh white-headed breed of the homozygous CC and VV genotypes of the CAPN1 and GH genes had 6.3% and 7.1% higher sum of unsaturated fatty acids in blood plasma, respectively, compared to the homozygous GG and LL variants. Predominance of unsaturated fatty acids over saturated fatty acids also determined the lower values of the index of lipid metabolism direction in the CC and VV genotypes: 0.92 and 1.00 versus 1.04 and 1.13 in the GG and LL genotypes (Table 1 and Table 2).

Table 1. Fatty acid composition of blood plasma lipids in young bulls of Kazakh white-headed breed of different genotypes, %

Fatty acids	CAPN1			GH		
	CC	CG	GG	VV	LV	LL
Saturated FA						
Myristic acid $C_{14:0}$	0.67	0.58	0.84	0.93	0.67	0.84
Pentadecanoic acid $C_{15:0}$	0.78	0.65	0.60	0.42	0.31	0.68
Palmitic acid $C_{16:0}$	22.12	22.81	23.02	24.21	21.67	22.99
Heptadecanoic acid $C_{17:0}$	2.69	2.49	1.91	1.83	1.40	2.15
Stearic acid $C_{18:0}$	21.81	24.11	24.51	22.51	25.82	26.33
Monounsaturated FA						
Oleic acid $C_{18:1}$	26.44	25.01	25.72	22.52	23.48	22.48
Heptadecenoic acid $C_{17:1}$	1.84	1.69	1.32	2.49	1.74	1.68
Polyunsaturated FA						
Linoleic acid $C_{18:2}$	17.35	16.97	16.01	19.25	18.23	17.01
Linolenic acid $C_{18:3}$	2.65	1.95	2.47	3.31	2.71	2.30
Arachidonic acid $C_{20:4}$	3.63	3.48	3.30	2.25	3.90	2.98

Table 2. The level of metabolites of energy metabolism in blood plasma in young bulls of Kazakh white-headed breed of different genotypes

gene	Genotype	Indicators						
		lipids, g/l	cholesterol, mmol/l	glucose, mmol/l	Sum of acids, %			lipid saturation index
					saturated	mono-unsaturated	poly-unsaturated	
CAPN1	CC	3.96 ±0.21	4.78 ±0.33	3.37 ±0.31	48.07	28.28	23.63	0.92
	CG	4.02 ±0.23	4.41 ±0.22	3.68 ±0.22	50.64	26.70	22.40	0.96
	GG	4.42 ±0.17	3.92 ±0.17	4.02 ±0.19	50.88	27.04	21.78	1.04
GH	VV	4.08 ±0.28	5.03 ±0.24	3.74 ±0.37	49.88	25.01	24.81	1.00
	LV	4.65 ±0,33	4.87 ±0.18	3.91 ±0.18	49.87	25.22	24.84	0.99
	LL	4.82 ±0.17	4.33 ±0.31	4.26 ±0.22	52.90	24.16	22.29	1.13

Comparative analysis of the content of total lipids, cholesterol and glucose in blood revealed that the total amount of lipids and glucose in blood was lower by on average 11.2% with on average 12.8% higher cholesterol level in the CC and VV homozygotes and the CG and LV heterozygotes of the CAPN1 and GH genes compared to the GG and LL homozygotes (Table 2).

Therefore, the obtained data indicate that the intensity of lipid metabolism in youngsters of Kazakh white-headed breed depended on a genotype by the CAPN1 and GH genes. It can be assumed that the carriers of the desirable C and V alleles of the CAPN1 and GH genes in the homo- and heterozygote state used the energy components of blood with higher intensity upon realization of the biosynthetic processes.

The revealed regularities were reflected in the value of live weight and average daily gains in different periods of ontogenesis. The carriers of the C allele of CAPN1 and the V allele of GH were superior at birth, weaning and 12 months of age. With that, the largest differences were found between the animals that were homozygous by these alleles and the young bulls, in which genotype they were absent. The difference on the 240th and 365th days was 13.8% ($P < 0.05$) and 6.9%; 14.6% ($P < 0.05$) and 6.9%, respectively (Table 3).

Comparison of the indicators of chemical analysis of *m. longissimus dorsi* revealed that the CC and VV genotypes were superior to other genotypes in the protein and fat content by 0.28–2.13 abs. percent.

Different protein and fat content in meat from animals of different genotypes was reflected in its caloricity. For example, the highest level of caloricity was observed *in m. longissimus dorsi* of the homozygous CC genotype of CAPN1 and the VV genotype of GH (118.9 and 122.5 kcal, respectively), which was 8.2% and 9.3% ($P<0.05$) higher than the similar indicator in meat of the homozygous GG and LL genotypes or on average by 8.7% (Table 4).

Table 3. Dynamics of live weight of young bulls of different genotypes by the CAPN1 and GH genes

Gene	Genotype	Quantity	Live weight, kg			Average daily gain, g
			at birth	at weaning, 240 days	365 days	
CAPN1	CC	6	28.1 ± 0.21	245.5 ± 4.6	374.2 ± 5.1	0.948 ± 0.92
	CG	12	27.9 ± 0.18	240.6 ± 6.5	368.9 ± 3.6	0.934 ± 0.72
	GG	75	27.6 ± 0.16	215.8 ± 5.2	349.9 ± 4.1	0.883 ± 0.85
GH	VV	29	28.3 ± 0.11	257.9 ± 7.5	382.7 ± 4.7	0.969 ± 0.65
	LV	17	27.9 ± 0.18	248.5 ± 4.5	373.8 ± 4.2	0.948 ± 0.92
	LL	47	27.6 ± 0.12	225.1 ± 6.1	357.9 ± 5.2	0.905 ± 0.71

Table 4. Chemical analysis of *m. longissimus dorsi* of young bulls of Kazakh white-headed breed of different genotypes by the CAPN1 and GH genes

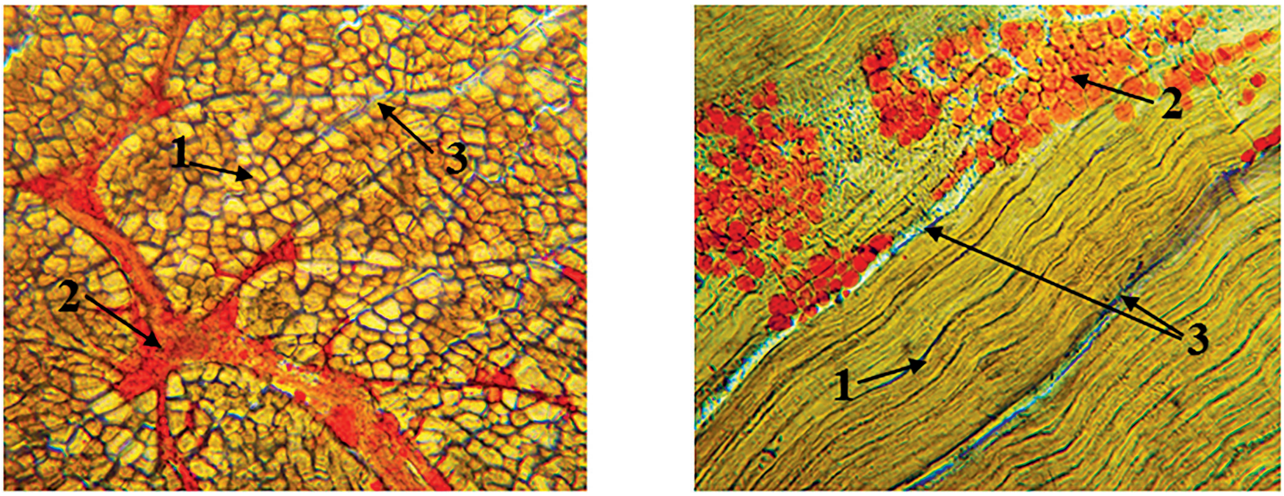
Indicators	Genotype					
	CAPN1			GH		
	CC	CG	GG	VV	LV	LL
Moisture, %	73.47 ± 0.27	74.90 ± 0.29	75.02 ± 0.18	74.37 ± 0.21	73.61 ± 0.17	74.84 ± 0.24
Dry matter, %	26.53 ± 0.16	25.10 ± 0.12	24.98 ± 0.15	25.63 ± 0.17	26.39 ± 0.09	25.16 ± 0.17
Protein, %	22.45 ± 0.11	21.49 ± 0.17	21.12 ± 0.14	23.12 ± 0.14	21.99 ± 0.17	20.99 ± 0.12
Fat, %	2.89 ± 0.04	2.47 ± 0.03	2.38 ± 0.03	2.98 ± 0.04	2.79 ± 0.04	2.60 ± 0.02
Caloricity, kcal	1189.1 ± 7.9	1111.4 ± 2.5	1099.2 ± 2.5	1225.3 ± 5.0	1179.6 ± 3.6	1121.4 ± 9.2

The highest quantity of muscle fibers was observed in the young bulls with the desirable CC and VV genotypes; the quantity was larger compared to the animals of the same age with the GG and LL genotypes in the CAPN1 and GH genes, respectively, by 36.82 fibers/mm^2 or 19.7% (P<0.01) and 55.7 fibers/mm^2 or 31.4%, on average 25.5% (P<0.01) and was significant (Table 5 and Figure 1).

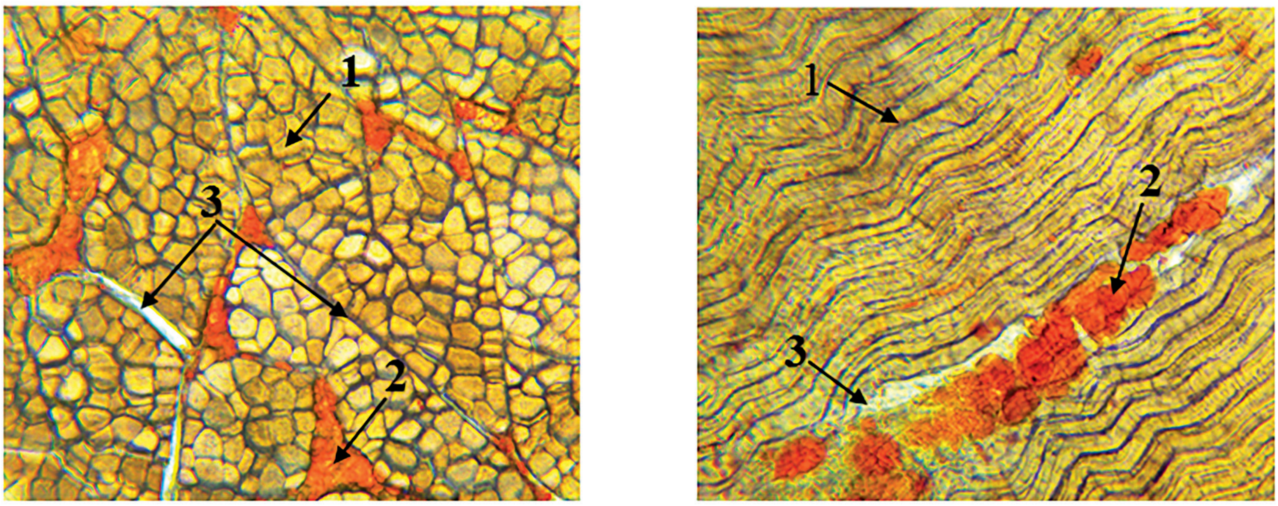
Table 5. Microstructural analysis of *m. longissimus dorsi* of young bulls of Kazakh white-headed breed of different genotypes by the CAPN1 and GH genes

Gene	Genotype	Indicators				
		Quantity of muscle fibers per mm^2	Diameter of muscle fiber, μm	Total score of marbling, points	Connective tissue content, %	Meatness, cm^2
CAPN1	CC	224.07* ±8.31	33.57* ±0.43	31.27 ±0.82	10.60 ±0.31	47.93 ±3.65
	CG	219.85 ±1.96	36.25 ±0.59	28.77 ±0.76	11.53 ±0.44	36.04 ±1.79
	GG	187.25 ±3.60	37.80 ±1.93	27.75 ±0.14	12.73 ±0.37	28.64 ±3.03
GH	VV	233.18* ±5.72	33.78* ±0.17	29.28 ±0.41	10.93 ±0.35	52.77 ±0.61
	LV	199.25 ±5.41	35.89 ±0.47	27.94 ±0.99	12.73 ±0.59	40.92 ±0.67
	LL	177.48 ±1.89	40.60 ±1.41	25.43 ±0.60	14.27 ±0.87	31.92 ±1.51

* P < 0,01



a)



b)

Figure 1. Histological sections of *m. longissimus dorsi* of young bulls of Kazakh white-headed breed of different genotypes by the CAPN1 gene
a) CC genotype b) GG genotype (left — cross sections; right — longitudinal sections)
1 — muscle fibers; 2 — fatty tissue; 3 — connective tissue (staining: Carazzi's hematoxylin with sudan III, 10 × 40 magnification)

The muscle fiber diameter was lower by 4.23 μm or 11.9% and by 6.82 μm or 16.8%, respectively, in the animals with the desirable genotype at high significance (P<0.001). Desirable genotypes also had an advantage in terms of the value of the marbling coefficient: the difference was 12.6% in the CAPN1 gene, 15.1% in the GH gene. At the same time, the content of connective tissue was higher by 2.13 and 3.34 abs. percent in the carriers of the undesirable G and L alleles in the homozygous state.

Conclusion

As a result of DNA typing, the frequency of desirable genotypes by the CAPN1 and GH genes among youngsters of Kazakh white-headed breed was established.

The carriers of the desirable CC and VV genotypes in the CAPN1 and GH genes had the largest live weight at the age of 8 and 12 months. Their superiority over the GG and LL genotypes was 13.8% and 6.9%, 14.6% and 6.9%, respectively.

The intensity of lipid metabolism in the young bulls of Kazakh white-headed breed depended on a genotype in the CAPN1 and GH genes. The carriers of the desirable alleles (C and V) in the homo- and heterozygote state in the CAPN1 and GH genes used the energy components of blood with higher intensity upon realization of the biosynthetic processes, which is indicated by their superiority in terms of the sum of saturated and unsaturated fatty acids and the index of lipid metabolism direction.

Muscle tissue of the animals of the CC and VV genotypes was characterized by the highest content of protein, fat and, respectively, energy value compared to the GG and LL genotypes. Meat from the young bulls of the desired genotypes had higher scores for marbling due to a larger quantity of interfiber fat inclusions.

Therefore, genotyping of youngsters of Kazakh white-headed breed by the CAPN1 and GH genes, selection of animals with desirable genotypes and their replication through the targeted selection of parent pairs will allow not only increasing meat productivity of a herd, but also will facilitate production of high quality beef.

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Review paper

APPLICATION OF HIGH HYDROSTATIC PRESSURE TECHNOLOGY TO IMPROVE CONSUMER CHARACTERISTICS AND SAFETY OF MEAT PRODUCTS

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Abstract
Recently, there has been a growing demand for healthy processed foods, such as comminuted or gel-type meat and fish products with reduced content of salt (sodium chloride), phosphate (sodium phosphate) and/or fat, while maintaining their texture and quality characteristics. As know, a high intake of dietary sodium is associated with cardiovascular diseases and strokes. On the other hand, high phosphate intake has a potential health risk, especially with regard to bone metabolism, cardiovascular and kidney diseases. High hydrostatic pressure (HHP) technology has been recognized as a useful method for successfully reducing salt, phosphate and/or fat content in processed muscle products. The texture, yield and organoleptic properties of products are closely related to the structure and functionality of myofibrillar proteins (MP). Application of moderate high hydrostatic pressure at 100–200 MPa has been successfully used to increase the functionality of myofibrillar proteins by modifying the structure due to denaturation, solubilization, aggregation or gelation. The ability to reduce sodium content and achieve a high binding and water retention using this technology is an important task for the production of healthy food products.

Introduction

The consumer demand for healthy food products has been steadily growing worldwide. With the increasing understanding of the link between nutrition and health the growth in changes in lifestyle and nutrition structure of consumers, the extension of knowledge about food processing, many processed foods products containing the high level of sodium and/or fat have been criticized due to the problem of their health effects. Healthy nutrition is understood by consumers as foods with different origin and composition; with that, the requirements for reducing edible sodium, fat and other food additives continue to be in the first place. The demand for healthy meat and fish products with reduced content of salt (sodium chloride), inorganic phosphate (sodium polyphosphate) and/or fat with maintenance of texture and taste characteristics of finished products has been growing.

Refusal or restriction of excessive consumption of foods contained saturated fats is linked with risks of obesity, development of chronic metabolic disorders and diseases of circulatory system [1,2].

High dietary sodium intake is associated with cardiovascular diseases and stokes [3,4]. In 2010, the global mean sodium chloride intake was 10.06 g/day [5]. The limit of sodium chloride intake recommended by WHO is 5 g/day. Japan and Russia Federation belong to countries with sodium intake above the recommended level (Figure 1). The

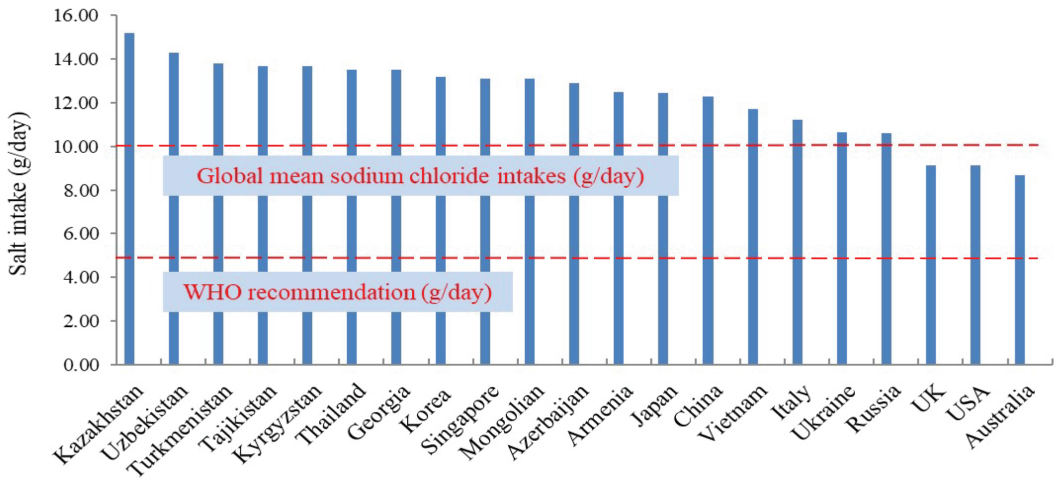

Figure 1. Countries with high sodium chloride intake. Adapted from Powles et al. [5]

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trend towards refusal of high sodium intake will allow a worldwide reduction in the risk of cardiovascular diseases, including strokes [3,4,6].

With that, humans have an increasingly active life, in which less time is available for food preparation at home. Processed foods became the main source of dietary sodium intake by modern humans — up to 77% of daily sodium intake comes with such products [7]. Moreover, processed meat products* account for 20–30% of daily sodium intake [8,9].

In processed meat products, sodium is contained mainly as sodium chloride. Its total amount in a product is built up due to its addition (1% to 5.5% of raw meat material weight) and its natural content in meat (less than 1%).

Another source of sodium in meat products is different sodium food additives — glutamates, phosphates, citrates, lactates, acetates and others. Among food additives used in the meat industry, food phosphates, which are used in an amount of up to 0.5% of meat raw material weight, should be highlighted as a significant sodium source. It is less than the addition of sodium chloride, which contains 39.7% sodium. However, food phosphates contain sodium in a comparable amount. For example, sodium tripolyphosphate (E451) contains 31.3% sodium and sodium pyrophosphate (E450) has 34.6% sodium [9,10].

From the perspective of healthy nutrition, processed meat products are also criticized for the unbalanced phosphorus (P) and calcium (Ca) ratio. Food phosphate additives cause concerns due to the sharp shift of the Ca: P ratio from the natural ratio of these elements in muscle tissue (1:5), which is per se far from the ratio recommended by dieticians (1:1). Due to added phosphate, the phosphorus content in the processed meat products is nearly twice as high as in the initial meat raw materials [11]. The potential effect of phosphate additives on population health can be still underestimated, especially in the conditions of low consumption of dairy products rich in calcium.

High phosphate intake has a potential health risk, especially, regarding bone metabolism, development of cardiovascular and renal diseases. Phosphate additives in processed food products can be harmful for population health due to the damage of vessels, provoke ageing processes, lead to the development of hyperphosphatemia, which was identified during the last decade as a strong predictor of mortality in humans with chronic renal diseases [12].

In this connection, consumers who prefer foods with the so-called "clean-label" perceive phosphates as undesirable food additives.

Besides meat products, phosphates are widely used in significant amounts in the production of fish products, baked foods, cheeses, carbonated soft drinks and so on [12]. Since the 1990s, the use of phosphate food additives increased from 500 mg/day to 1000 mg/day, which indicates the topicality of the development of technologies that does not envisage their use [13].

Different countries of the world undertake legislative measures that guarantee the presence of healthy food products on the market for consumers. Similar measures are taken in Japan and the Russian Federation. For example, since 2018, the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) has been realizing the project of voluntary food product labeling "traffic light", which envisages color coding on product labels: green, yellow and red depending on the content of salt as well as sugar, fat, saturated fats, energy value (caloricity) with consideration for daily intake. The main task of this labeling is to give consumers visual information, which will allow them to make a correct choice when buying food, as well as facilitate adherence to the principles of healthy nutrition and reduction of risks for population health [14].

Since 2019, Rospotrebnadzor has been implementing measures aimed at propaganda of healthy nutrition principles and the creation of conditions in the Russian Federation that will facilitate the formation of healthy lifestyle in the framework of the Federal Project "Strengthening Public Health". The Federal Project presupposes the introduction of a system for monitoring nutrition of different population groups, including children, in regions, which will allow establishing a connection between the nutrition structure, food quality and population health state. The monitoring system will enable the accumulation of data that are necessary for assessing actual nutrition in different regions of the country and their use for developing targeted educational programs and updated recommendations on nutrition for different population groups [15].

This year, the Ministry of Health of the Russian Federation published the order No. 8 of January 15, 2020 "On approval of the strategy for the formation of a population healthy lifestyle, prevention and control of noncommunicable diseases for the period up to 2025". According to the strategy, one of the main measures of noncommunicable disease prevention is rational nutrition including daily consumption of fruit and vegetables (not less than 400 g/day), reduction of free sugars (to a level of less than 10% total energy intake, which is equivalent to 50 g/day), consumption of fats in an amount of less than 30% of total energy intake, consumption of salt less than 5 g/day [16].

On the other hand, Japan has been implementing the targeted national 10-year program on improving health in the 21st century ("Health Japan 21"). The program was launched in 2013 and updated in 2018. According to the basic "Health Promotion Act", the program is aimed at the reduction of premature deaths, the extension of healthy life expectancy and improvement of life quality. Much attention is paid to the creation of environment for the prevention of lifestyle-related diseases and the assurance of food safety. In Japan, the Ministry of Agriculture, Forestry and Fisheries (MAFF), which is responsible for production

* here and elsewhere this expression means meat and meat products that have not only raw meat materials in their composition.

and assurance of food quality, and the Ministry of Health, Labour and Welfare (MHLW), which regulates the stability of distribution and safety of foods, are together engaged in solving these tasks at all stages from production to consumers.

To stimulate an improvement in nutrition habits of the population, MHLW establishes and revises every five years Dietary Reference Intakes for Japanese. The basis of these recommendations is constant monitoring of the latest scientific achievements and international standards, as well as the analysis of the actual results of the annual National Health and Nutrition Survey in Japan. A consideration of the peculiarities of the traditional cuisine and lifestyle in developing recommendations results in several differences with the recommendations of the Codex Alimentarius Commission [17]. In particular, salt intake in Japan is significantly higher than the norm of 5 g recommended by WHO, although it has been constantly reduced. In recommendations of 2015, salt intake was established at a level of less than 8 g per day for males and 7 g per day for females. The last edition of 2020 recommends the intake of less than 7.5 g per day for males and 6.5 g for females. The daily intake of fats at a level of 25% of total energy intake is recommended [18,19].

To prevent lifestyle-related diseases, the Guidelines for a balanced diet (from the Ministry of Agriculture, Forestry and Fisheries) recommend increasing consumption of vegetables up to 350 g and more, and fruit up to 200 g and more per day [20]. A strong emphasis is placed on the education of schoolchildren to increase familiarity of the nation with the issues of safety and balance of food products.

Since 2015, food labeling has been carried out according to the Food Labeling Act of 01.04.2015 with Consumer Affairs Agency (CAA) controlling its execution.

The Food Labeling Act applies to all food types (including food additives) and beverages (including alcoholic) with the exception of pharmaceutical drugs. The specific requirements for the label content significantly increased and are put down in the Food Labeling Standards. Labeling such components as the energy value, content of proteins, fats and carbohydrates, as well as salt equivalent (total sodium content) became mandatory. The mandatory requirements for labeling of the main allergenic foods and additives, the presence of L-phenylalanine compounds were introduced. From March 2022, marking countries of raw material origin on a label will become mandatory, even for the finished products finally packed or processed on the territory of Japan.

The inclusion of the content of saturated fats and dietary fibers into information for consumers that is placed on a label is voluntarily but recommended. Labeling of available carbohydrates, sugar, cholesterol, vitamins and minerals is completely voluntary [21].

Requirements for labeling food with health claims are different for each of the three categories. Products containing food components with proven effects on the physiological functions of the human body (Food for Specified Health Use; FOSHU) require mandatory control, registration in the state bodies — and have special labeling. Products with the declared food value (Food with Nutrient Function Claims: FNFC) have to account in labeling the recommended daily intake (for example, for vitamins and minerals) and are based on state standards. The most flexible category includes products with stated functional properties (Foods with Function Claims: FFC) and was introduced in 2015. The corresponding marking on a label is done according to available scientific research about the health benefits of an ingredient and is a responsibility of a producer. For any category, permission for placing information about one or another physiological effect from a product is given only after research confirming this effect and misleading consumers with false statements is punishable by law [21].

Therefore, food legislation in different countries set new tasks for the meat industry regarding further development of the functional product market and widespread reduction/exclusion of using undesirable components of meat products, first of all, salt, phosphates and/or fat. At the same time, sodium chloride and sodium phosphates are the main recipe components of meat products having an important function — extraction of myofibrillar proteins (solubilization) and formation of meat gel, which holds water, stabilizes fat in a product and form product consistency. Solubility, water-holding, gelation and emulsification of myofibrillar proteins depend on salt and phosphate addition and these functional and structural protein properties are important determining factors of consumer quality of meat products (appearance, color, texture, taste, aroma) as well as economic indices (yield and cost of finished products).

Processed meat products traditionally continue to be quite popular among consumers, but their assortment includes an insufficient quantity of products with low content of salts, phosphates or fat on markets in different countries. In other words, there is a growing demand for healthy, nutritional and economical muscle products with low content of food additives and maintenance of their textural and taste attributes. Nevertheless, reduction of the content of sodium salts or fat creates significant problems for manufactures of the meat industry and negatively influences such product quality characteristics as color, texture, organoleptic indices, as well as yield and primary cost of finished products. While a certain rise in product prices can be accepted by a consumer, maintenance of familiar sensory characteristics when excluding/reducing undesirable recipe components is the main problem of healthy nutrition products. Consumers will always want not only healthy but also tasty food. This makes scientists to search for new technical and technological solutions to create products of healthy nutrition with the maintenance of the required quality level. With that, in addition to meeting requirements for the composition and sensory characteristics, requirements for safety and shelf-life of meat products are progressively increasing.

Over the last years, several approaches to the reduction of the sodium or fat content in processed meat products have been formed, among which are:

- use of salt replacers that exclude/reduce sodium intake, in particular, potassium chloride (KCl), in combination with substances masking off-taste;
- use of taste enhancers that increase the perception of product saltiness when used simultaneously with reduced salt amount;
- optimization of the physical form of added salt that allows enhancing and/or creating salty taste in a particular moment of product consumption (reduction of salt crystal sizes, formation of double emulsions);
- replacement of fat with hydrocolloids;
- use of special technologies for raw material processing (for example, processing with high hydrostatic pressure) [22,23,24,25,26,27,28,29,30,31,32,33,34,35].

Nowadays, in the Russian Federation, the main measures regarding sodium content reduction in processed meat products are directed towards decreasing doses of edible salt addition and the use of its replacers, which, in turn, cannot ensure clean-label [32].

There is an urgent need for searching alternative methods that will allow manufacturing products with low sodium and/or fat content and high functionality. Today, physical methods of processing have been finding increasingly wider application in the world and are regarded as promising technologies that enable a significant reduction of industry demand for food additives.

High hydrostatic pressure (HHP) pre-treatment at 100–200 MPa before heat treatment is one of the methods for successful reduction of the content of salt, phosphate and/or fat in gel-type or minced meat and fish products.

It was shown that the use of high pressure impacts myofibrillar proteins (MP) similar to the effect of salts; consequently, both sodium chloride and sodium phosphates can be reduced by this method [36]. High pressure (HP) is used for increasing the functional properties of MP, causing structural modifications, leading to denaturation, aggregation and gelation. Improvement of functional properties by enhancing water-protein and protein-protein interactions changes the textural properties, improves water binding and increases stability of the meat system. These modifications depend on a product composition, introduced food additives, pressure parameters, pressure/temperature combinations and the sequence of application [37].

The above mentioned aspects determined the aim of this review: (1) to study the main details of structural and functional changes in myofibrillar proteins (MP), modified by high hydrostatic pressure (HHP); (2) to present an overview of achievements in the field of using high hydrostatic pressure (HHP) technologies to produce meat products of healthy nutrition with improved functionality and increased shelf-life.

Main part

Use of high pressure technology in the food industry

The first use of high pressure (HP) in the food industry was proposed by Hite in 1899 for the pasteurization of milk and fruit products [38]. However, at the end of the 1980s, it was Japan that became the first country using a hermetically sealed vessel specially designed for carrying out the HP procedure in the food industry to produce jam and fruit puree for commercial purposes. Since the first application in Japan, this technology was significantly developed with respect to technological aspects and assortment of pressurized food products on sale [39].

High pressure processing is a non-thermal technology in which a product is treated with a pressure of 100 MPa and higher using compression fluid [40]. There are three forms of such processing: a static process in a vessel at high hydrostatic pressure (HHP) during a certain time and at the certain temperature; the dynamic process in which fluid is pressed through a nozzle jet under high pressure (homogenization by high pressure); hydrodynamic processing with pressure or shocking waves which instantaneosly development pressure waves up to 1 GPa [41].

The high pressure technology is based on two principles that determine the behavior of a product under pressure. An important principle underlying the effect on reaction equilibrium is known as the Le Chatelier's principle, according to which any phenomenon that is accompanied by volume reduction will be enhanced by a HP increase and vice versa. The use of HP leads to physical and chemical changes. However, chemical changes are minimal; HP does not affect covalent bonds, influencing only weaker ionic and hydrogen bonds; that is, hydrophilic and hydrophobic interactions. As HP does not impact covalent bonds, the majority of low molecular weight compounds such as vitamins and flavor substances do not change. Nevertheless, HP affects high molecular weight components of food and can cause protein denaturation and aggregation which leads to a change in raw material texture both with a positive and negative result of such the effect on final product quality [39]. HP significantly influences product microflora. Microbial inactivation that facilitates food preservation is a result of cell membrane damage, protein denaturation, changes in intracellular enzyme activities, ribosome damage, and other effects caused by HP [42].

The second important principle that explains the effect of HP is isostatic. According to this principle, the pressure is instantaneously and uniformly transmitted throughout a product volume without a gradient. Therefore, the HP is characterized as an isostatic quantity. Pressure does not depend on a product size or geometry, and pressure transmission to the product center does not depend on its mass and process duration. After pressure treatment, a product returns to its initial shape [39].

Programs on processing foods, beverages and other products (Figure 2) began to be performed from the 1990s, and one of the first works was a shelf-life extension of

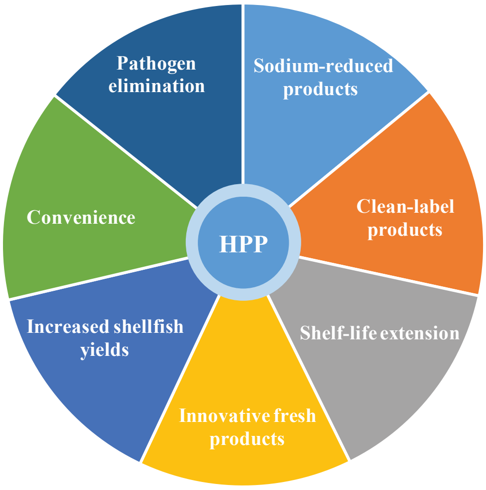

Figure 2. Practical uses of HP pasteurization [43]

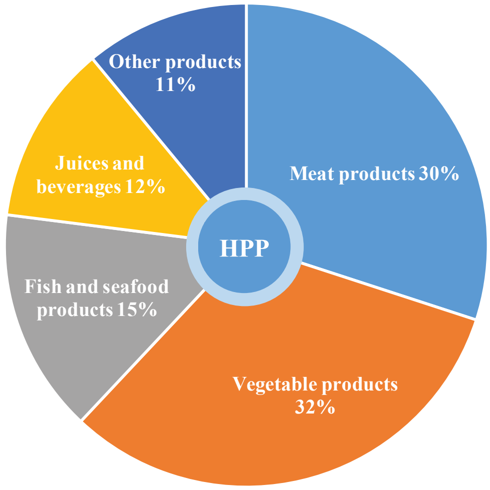

Figure 3. Distribution of HP equipment in food industry [45]

avocado-based products. This processing method leads to an effective reduction of the microbial contamination with minimum impact on the sensory characteristics and nutritional value. Very soon, the HP technology became known as a post-packaged preservation technology for ready-to-eat products: whole or sliced meat products, seafood, fresh-cut fruit and juices, as well as salads, sauces, dressings, soups and so on [36,43].

Today, more than 160 industrial units (vessels) are used worldwide [39]. A large part of the equipment (44%) is intended for processing products based on fruit and vegetables (Figure 3). It is explained by the fact that avocado processing was one of the main drivers of this innovative technology [44,45]. In addition to microbial inactivation at low temperatures, this technology can be used to develop new products without thermal impact or for manufacturing similar products with minimum effect on taste, color and nutritional value [40].

High hydrostatic pressure (HHP) processing had received wide recognition in the meat sector. It is, possibly, linked with the fact that meat products have a high commercial value which allows investing and covering expenses on such processing. At present, 25–30% of the whole volume of HP equipment is used in the meat industry [36]. Over the last decades, the technology has been widely used in meat product manufacture for inactivation of pathogenic microorganisms and, therefore, for shelf-life extension [46]. However, the HP technology is not regarded any more as a simple alternative to conventional pasteurization. Today, this technology is increasingly often used as a processing method that causes structural modifications to create innovative meat products with increased functionality, products with reduced content of food additives, products with a modified structure for certain population groups [37]. Therefore, HP continues to be an alternative to heat treatment to ensure microbiological safety and is used to change a product structure with maintenance of its nutritional value. This ensures the high potential for modification of the assortment of minced meat products, creation of innovative products by influencing the structural and functional properties of myofibrillar proteins (MP). This can facilitate a reduction in the use of food additives with E-numbers and an increase in manufacturing products with clean-label [37].

Researchers [29,47,48,49,50,51] showed that the use of HHP in meat processing to improve functional properties of myofibrillar proteins opens wide possibilities for production of meat products with reduced content of salt, phosphate, fat and calories. HHP can be used to change the structure of MP modifying protein by disrupting non-covalent interactions (electrostatic and hydrophobic) in tertiary and secondary structures within protein molecules and the subsequent formation of intra/intermolecular bonds. The control of pressure-induced structural and functional changes in MP in meat systems is an important question for meat product manufacturers [37].

The result of structural modification of MP depends on inherent characteristics of a product (species, type, pH, composition, presence of one or another ingredient) and conditions of HHP application (pressure intensity, pressure gradient, duration, temperature, pressure/temperature ratio, sequence of stages of technological processing and pressure application) (Figure 4). The corresponding choice of HHP parameters can change meat quality, functional characteristics of muscle proteins and, partially, compensate a decrease in doses of salt and food additive introduction and in the fat content, thereby, increasing the meat product value [37,52,53].

Effect of high pressure on the meat structure

As known, a muscle contains three types of proteins classified by their solubility and location in muscle tissue — sarcoplasmic, myofibrillar and stroma proteins. Myofibrillar proteins (MP) or salt-soluble proteins, which are soluble only in solutions with relatively high ionic strength (>0.3 M NaCl or KCl) account for about 50 to 56% of the total quantity of muscle proteins and are linked with the meat structure. MP extracted from a muscle fiber, mainly consist of myosin and actin with myosin as the most abundant protein. Myosin is the major MP which is

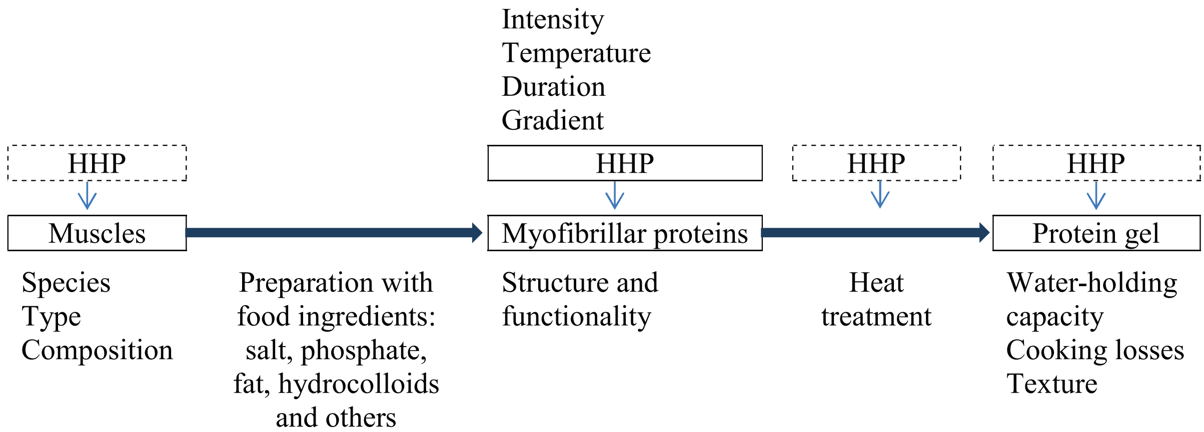

Figure 4. Schematic presentation of processes of technological processing of meat products using HHP and formation of meat product characteristics. Adapted from Chen et al. [37]

Note: HHP — high hydrostatic pressure

responsible for the total solubility, emulsification, gelation, water- holding. These functional properties are important determining factors for yield, texture and organoleptic indices of meat products.

One of the main effects of HP impact on meat proteins is depolymerization. It was confirmed that depolymerization of F-actin, extracted myosin and actomyosin occurs at the pressure intensity of 100 to 300 MPa. It is assumed that depolymerization facilitates an increase in solubility of MP [36,37,54].

Pressure-induced structural changes in myofibrillar proteins were also well studied. MP began unfolding under the pressure of 50 to 300 MPa, and higher pressure levels lead to increased denaturation, agglomeration and gelation. These structural modifications of meat proteins under pressure can be used in developing products to affect structure formation and/or water retention. Also, pressure-induced structural changes in myosin head (myosin subfragment-1, S-1) were described at pressure from 150 MPa [36,37,55]. Therefore, pressure can cause partial or full unfolding, denaturation, reversible or irreversible aggregation of MP.

One of the significant effects of HP on meat proteins is the modification of actin-myosin complex. Suzuki et al. (1990) studied the effect of HP (100–300 MPa) on beef post-rigor muscle and noticed that maximum fragmentation was achieved at 300 MPa within 5 min. In addition, the Z-line in myofibrils was not apparent in pressurized muscles [56]. Iwasaki et al. (2006) also described the destruction of the M-line and Z-line, and dissociation of thick and thin filaments within myofibrils under HP. This arrangement of the thick and thin filaments within myofibrils is mainly responsible for the ability to retain water in muscle cells. Therefore, any spatial rearrangement or disruption between filaments affects the ability to retain water [48].

It was noticed that HP pre-treatment changed the conformation and functional properties of MP which subsequently can influence the gelling property of protein upon heat treatment. Heat-induced gelation of myosin is a key factor in the production of minced meat and fish products (pastes and so on). The HP effect on denaturation and gelation of meat proteins significantly depends on many factors such as a protein system, ionic strength, presence of additional ingredients, and parameters of HP application. Treatment of MP with pressure higher than 100 MPa at low temperature causes protein solubilization and unfolding which are necessary for initiation of protein gelation. However, both the conformation condition of myosin and salt content can influence the gelation of myofibrils. HP-induced gelation of myosin chains occurs as a result of their interaction with each other similar to heat-induced gelation; however, tail to tail interaction of the protein structures is not observed when HP is applied [57,58,59,60]. Therefore, HP-induced meat gels are often weaker than heat-induced gels. Nevertheless, the use of HP pre-treatment at a low temperature before heat treatment can enhance the thermal gelation of myosin (Figure 5). Thus, myosin pre-treated with HP retains its ability to form aggregated structured gel as a result of heat treatment (after heating and cooling) [59].

For example, Macfarlane et al. (1984) showed that HP processing at 150 MPa for 10 min at 0 °C improved heat-induced (70 °C, 10 min) gelation of myosin from sheep muscle at low ionic strength [61]. They also reported that HP pre-treatment (150 MPa, 20 min, 0–3 °C) increased structural-mechanical characteristics (tensile strength) and water-holding capacity of cooked beef patties contained 1% NaCl.

Iwasaki et al. (2006) also suggested that depolymerization of thin filaments was a cause of high apparent elasticity of heat-induced myofibrillar gels when using HP pre-treatment at 200 MPa. Pressure processing at 200 MPa (10–20 min, 20–25 °C) before heat treatment at 70 °C increased the elasticity of pork patty contained 1% NaCl (at pH 6) and chicken myofibrillar gel by 2–3 times, respectively [48]. They suggested that HP processing at 300 MPa causes shortening of myosin filaments, which can facilitate a decrease in elasticity of heat-induced myofibrillar gels compared to pressurized gels.

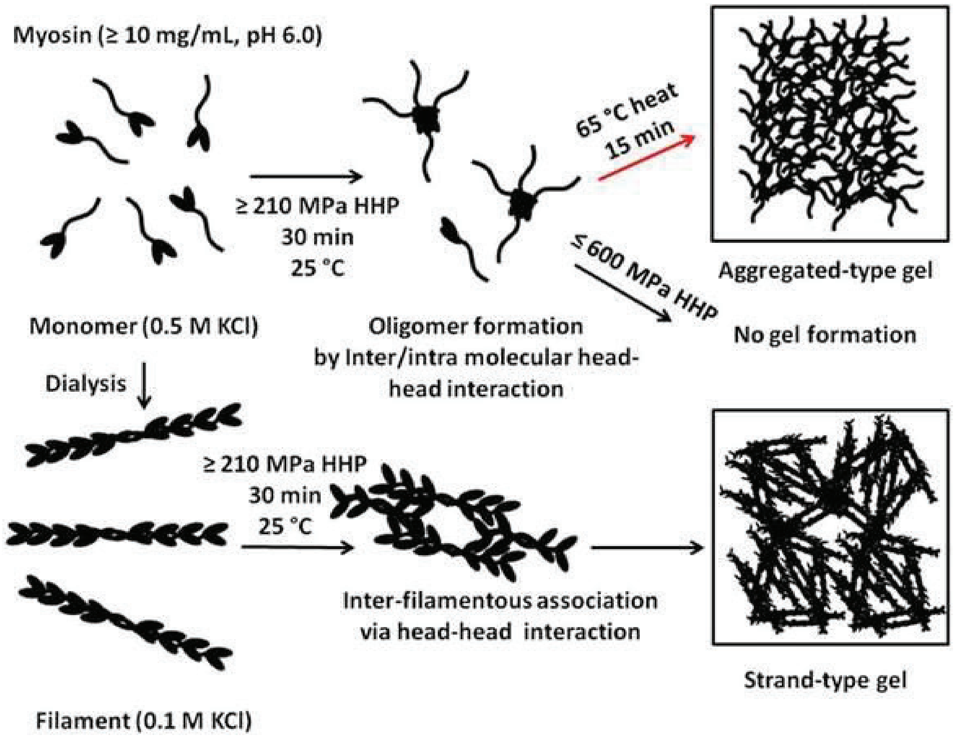

Figure 5. Proposed mechanism of the HHP-induced aggregation and gelation of myosin in high ionic strength (0.5 M) or low ionic strength (0.1 M) solution at pH 6.0 based on the findings of Iwasaki et al. (2005); Yamamoto et al. (1990); Yamamoto et al. (2002) and Yamamoto et al. (1993) [57,58,59,60].

Note: HHP — high hydrostatic pressure

Ikeuchi et al. (1992) suggested that improved gelation of actomyosin induced by HP pre-treatment is explained by denaturation of actin in actomyosin and increased content of sulfhydryl, as well as increased surface hydrophobicity. As shown in Figure 6, the high quantity of F-actin negatively affects thermal gelation of actomyosin at low and high KCl concentrations according to the theory proposed by Yasui et al. (1980) [62]. HP pre-treatment at 100–150 MPa will effectively reduce a negative effect of excessive quantity of F-actin in actomyosin on gel strength of myosin via depolymerization of actin. As a result, HP pre-treatment at 150 MPa increases the heat-induced gel strength of actomyosin [63].

The initial effect of HP impact on MP consists in dissociation of the quaternary structure causing protein unfolding and solubilization, and exposing functional groups of the protein surface. As shown in Figure 7, treatment at moderate pressure levels (100–200 MPa) increases the solubility of MP due to complex dissociation and depolymerization of the protein structure. MP denature and moderately stretch causing destabilization of their protein structures and exposure of hydrophobic and sulfhydryl groups. During the following heating, the helical tail portion of myosin unfolds and multiple cross-links of the tail-tail type are formed for water holding. This leads to the formation of the regular, homogeneous, filamentous, three-dimensional network with increased water-holding capacity (WHC) and gel strength.

On the contrary, treatment with pressure higher than 200 MPa causes strong protein-protein interaction with

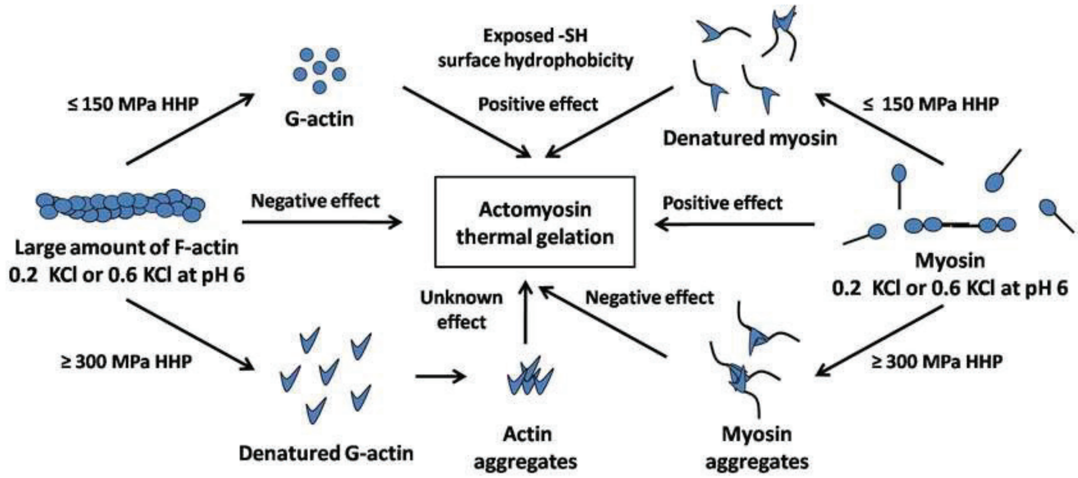

Figure 6. Proposed mechanisms on the effects of HHP on the thermal gelation of actomyosin in 0.6 M or 0.2 M NaCl/KCl at pH 6.0 based on the findings of Wang et al. (2017); Cao et al. (2012); Iwasaki et al. (2006); Ikeuchi et al. (1992) and Yasui et al. (1980) [48,62,63,64,65,66].

Note: HHP — high hydrostatic pressure; SH: sufhydryl groups

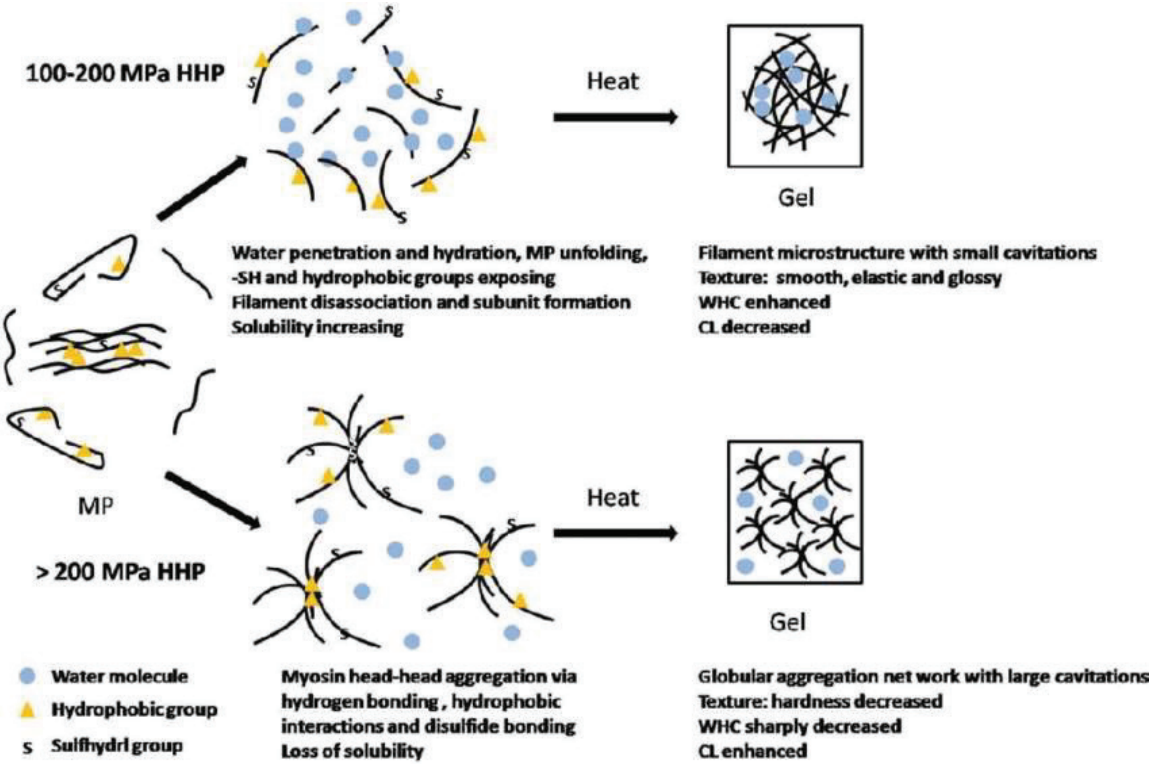

Figure 7. Proposed mechanism of HHP impact on denaturation, solubilization, aggregation and thermal gelation of MP in 0.6 M NaCl/KCl at pH 6–7 [64]

Note: HHP — high hydrostatic pressure, WHC — water-holding capacity

the participation of hydrophobic radicals, hydrogen and disulfide bonds forming large insoluble aggregates. When heating, the α-helical structure of the tail portion of myosin unfolds to a lesser extent. Water-protein interactions during association of myosin tails are suppressed and protein-protein interactions will advance. As a result of processing with pressure higher than 200 MPa, the globular aggregated network will be formed, which is characterized by cavities of a larger size between protein chains. Such segmentary structure will be weakened and cannot hold water effectively.

Therefore, when it is necessary to increase the WHC of protein gel after heat treatment, pressure of not more than 200 MPa is to be applied.

Nevertheless, salt and phosphate concentrations should be regarded as key factors influencing gelation under HP. It is assumed that the presence of 1% salt can increase the sensitivity of meat proteins to an impact of HP. Similar to the effect of meat protein denaturation, it depends on the intensity of pressure application and on the presence or absence of salt and phosphate in meat systems [37].

Use of high pressure technology to develop healthy, functionally improved gel-type or minced muscle products

Recently, the HHP technology was tested in several studies on different meat and fish products to satisfy consumer requirements regarding the reduction of salt, phosphate and fat content (Table 1).

Studies on the application of HHP pre-treatment of beef, pork and fish proved the applicability of this technology for the production of meat products with reduced sodium content. Sikes et al. [47] treated the beef batters contained 0%, 0.5%, 1%, and 2% salt at different pressure parameters (0.1–400 MPa) at 10 °C, for 2 min. They observed that beef sausages treated at 200 MPa and contained 1% salt had texture similar to sausages with 2% salt. With that, the samples of sausages had the same cooking losses. Thus, it was shown that HHP use leads to an increase in yield and improvement in finished product texture. The researchers suggested that improved WHC of low salt batters was determined by increased the solubility and gelling property of protein as a result of its partial unfolding under an effect of HHP treatment.

Maksimenko et al. [50] studied the effect of HHP treatment (100–200 MPa, 10 min, 20 °C) in combination with the sodium chloride (0–2%) and sodium phosphate (0–0.5%) addition on the physico-chemical properties of beef gels (color, WHC, protein composition, texture). Beef gels pressurized at 150–200 MPa showed the synergetic effect in the increasing water content and the reducing cooking losses compared to unpressurized beef gels. The L*, a* and b* color values of raw beef gels slightly decreased upon HHP treatment; however, HHP-induced color changes in raw beef gels did not significantly influence the color of beef gels after heat treatment. Electrophoretic investigation of samples by SDS-PAGE showed that the staining intensity of the α-actinin protein band decreased in the HHP-treated beef gels. The cohesiveness, adhesiveness, gel strength and modulus elasticity of beef gels with low concentration of sodium chloride and/or sodium phosphate after heat treatment were improved as a result of HHP application

Table 1. Results of the studies on HHP application to develop new products with reduced content of salt, phosphates and/or fat

Product	Conditions of the experiment	Result of the experiment	Proposed mechanism	Reference
Low fat and low salt beef sausage	Low fat beef mince was prepared with 0.6%, 0.8%, 1% NaCl and then HHP processed at 0.1–400 MPa/10 °C/ 2 min	Samples with 1% NaCl and treated with HHP at 200 MPa had similar texture characteristics and cooking losses compared to samples with 2% NaCl and without HHP treatment	An increase in solubilization of myosin and actin, improvement in binding of muscle components	Sikes et al. [47]
Low fat and low salt beef gel	Low fat beef gel was prepared with 0–2% NaCl and 0–0.5% phosphate, and HHP pretreated at 0.1–200 MPa/20 °C/10 min	Texture and moisture binding capacities were improved after HHP treatment at 150 MPa	Combined use of moderate HHP and low salt concentration caused modification of protein structure, reduction in concentration of α-actinin and M-protein, improvement in water-protein and protein-protein interactions	Maksimenko et al. [50]
Pork sausage with reduced salt and phosphate content	HHP pre-treatment of mince at 0.1–150 MPa/20 °C/5 min before mixing with 0.5–2.5% NaCl and 0.25% phosphate	HHP treatment at 150 MPa can be used for reduction of salt content up to 1.5% while maintaining acceptable organoleptic and functional properties	—	O'Flynn et al. [29]
Pork sausage with reduced phosphate content	HHP pre-treatment of mince at 150 and 300 MPa/20 °C/5 min before mixing with 0%, 0.25% or 0.5% phosphate	HHP treatment at 150 MPa has potential for phosphate reduction (0% or 0.25%) without significant changes in functional and consumer properties	HHP caused solubilization and extraction of MP without using food additives, modified protein structure, facilitated binding of meat particles	O'Flynn et al. [51]
Fish (surimi) gel with reduced salt content	Fish gel was prepared with 0.3% NaCl or 3% NaCl and HHP treated at 0.1, 150, 300 MPa/10 °C/10 min	Mechanical and organoleptic characteristics of gel with 0.3% NaCl were improved after applying HHP at 300 MPa and showed the same results compared to gel with 3% NaCl	HHP induced protein denaturation in low salt samples is similar to unfolding of protein structures in the presence of high salt concentration (3%)	Cando et al. [67]

at 150–200 MPa/ 20 °C/10 min. The authors came to the conclusion that the combined use of the low sodium chloride concentration and HHP at 150–200 MPa before heat treatment allows reducing salt concentration up to 1% with simultaneous maintenance of sensory and texture characteristics. They suggested that an improvement in texture properties and maintenance of WHC in the pressurized samples containing 1% salt is caused by changes in the protein fractional composition: losses of M-protein and aggregation of α-actinin.

O'Flynn et al. [29,51] reported that low salt sausages (salt content reduction from 2.5% to 1.5%) can be produced using meat pretreated with HHP at 150 MPa, 20 °C. These parameters allowed maintaining familiar sensory and functional properties of sausages. Moreover, the use of HHP processing improved rheological and texture properties, reduced cooking losses.

HHP pre-treatment can be successfully adapted to obtain low phosphate and low fat meat products. This was confirmed by studies that showed that HHP caused solubilization of myofibrillar proteins without the additional introduction of phosphate additives. Changes in the protein structure caused by HHP allow obtaining minced meat products with improved functionality [37]. The use of HHP processing at 150 MPa for 5 min. has a large potential for reducing phosphate levels up to 0.25% in low fat sausages without significant changes in their functional characteristics. The authors [53] reported that sausages with 0.25% phosphate content had improved firmness, adhesiveness, cohesiveness, and consistency, when pork meat batters were processed at 150 or 300 MPa/20 °C/5 min, compared to sausages with 0.5% phosphate content that were not treated with high pressure.

Maksimenko et al. [50] also reported that beef gels prepared with 1% NaCl without the addition of phosphate and fat that were HHP pre-treated at 150 MPa/20 °C/10 min, demonstrated improved texture and functional characteristics after heat treatment compared to the unpressurized samples with 2% salt + 0.5% phosphate content. In cooked sausage production without phosphates addition, pressurization at 100 MPa/20 °C/5 min improved functionality of meat proteins and allowed obtaining a product with the corresponding structure [36].

Based on similar considerations, HHP processing was used for development of gel-type fish products. Cando et al. [67] found that HHP processing at 300 MPa/10 °C/10 min improved the mechanical and sensory properties of gel with reduced salt concentration (0.3%) and stabilized protein structures in these gels to the same extent as in the samples with 3% salt content. The process of gel formation with participation of muscle tissue proteins from fish and slaughter animals is different; thus, conditions of HHP exposure are not the same.

Therefore, investigations carried out on various raw material types showed the possibility of managing the functional properties of muscle proteins. However, not only physical parameters are important in HHP technology. Along with them, salt and phosphate concentrations should be regarded as the main factors influencing the gel formation of meat proteins. In other words, prudent combination of recipe selection and HHP technology allows significant reduction in used salt and phosphate food additives.

Use of high pressure technology to increase safety and maintain quality of meat products

Another important direction for the application of HHP technology is an? increase in the microbiological safety of meat products. This direction considers the use of significantly higher ranges of HHP than parameters recommended for solubilization and the increase of functional characteristics of MP.

Several studies [68] showed that exposure of meat products with the HHP of up to 600 MPa for 3 min can inactivate dangerous pathogenic microorganisms (L. monocytogenes, E. coli, Salmonella and others), as well as the majority of mold species. To suppress vital activities of molds, treatment at 400 MPa is considered sufficient; however, it is noted that higher HHP level and longer exposure are required for killing yeast spores [69]. Effectiveness of HP treatment of meat products depends on existing microbial species, chemical composition, pH, and water activity of a product. HHP over 800 MPa causes the destruction of microbial cell membranes and intracellular protein structures leading to the disorder of homeostasis and cell death. The application of HHP in combination with other types of technological processing (blanching, dehydration, roasting, freezing-thawing, and others), opens wide possibilities for creating combined technologies to improve microbiological safety and product stability [69].

pH values higher than 6.2, which are typical for DFD meat, on one hand, ensure high functional characteristics of raw meat material, and, on the other hand, prevent predicting a possibility of its long term storage in the chilled form. Investigations of chilled DFD beef samples treated with HHP at 800–1000 MPa for 5 min and stored at a temperature of 0 to +4 °C in a vacuum package showed that samples had high sensory characteristics that corresponded to fresh DFD meat after 60-day storage. With that, microbiological indices in HHP-treated beef were lower than the normative values even after 60 days of storage [70].

HHP treatment at 800 MPa for 5 min. increased the sanitary-hygienic condition of chilled beef packed under vacuum in a polymer package 48 hours after slaughter and also prevented protein decomposition and facilitated retardation of fat oxidation processes in meat. The beef samples corresponded to the regulatory requirements for microbiological safety after 60-day storage [71].

Investigations of the microbiological indices of mechanically deboned chicken meat treated with HHP (250 MPa for 15 min.) during storage at a temperature of 4±2 °C showed the possibility of the two-fold increase in product shelf-life compared to control samples not treated with HHP. It was observed that HHP treatment did not affect the nutritional value of the product, and sensory indices corresponded to the initial characteristics. It was shown that HHP treatment used for raw meat materials is highly technological and easily incorporated in the process of meat product manufacture. With that, HHP at 250 MPa allowed increasing not only sanitary indicators but also functional-technological characteristics of raw materials [72].

HHP can significantly retard oxidative processes, which occurrence can significantly influence meat and meat product storability. The research [70] showed that processing at 800 MPa and 1000 MPa enabled reducing the peroxide values in chilled beef samples by 9 and 4.5 times compared to untreated samples. However, an increase in pressure of up to 1000 MPa was undesirable as oxidative processes in a product enhanced compared to the samples treated at 800 MPa. During storage of packed chilled beef for 30 and 60 days, the samples pressurized at 800 MPa had the peroxide value on the 30th day two times lower and on the 60th day four times lower than in the unpressurized samples [70].

The research [71] also showed low growth dynamics of the acid value and peroxide value in the samples of beef shoulder packed in a polymer package in 500 g pieces and pressurized at 800 MPa for 5 min. The acid value and peroxide value in pressurized beef on the 60th day of storage were six and four times lower, respectively, compared to the control sample.

HHP treatment of chilled meat activates its natural antioxidant systems, in which an important role is given to such compounds as vitamin E, polyunsaturated fatty acids and several amino acids (histidine, leucine, threonine, valine, and glutamic acid), and others. During HHP impact on muscle tissue, the lysosome membranes containing enzymes are destroyed and cathepsins are actively released. The process of enzyme release accelerates autolysis and amino acids with antioxidant properties are actively formed, which explains the enhancement of meat antioxidant properties [73].

Vacuum packed meat processed with HHP at 800 MPa is less subjected to changes in protein substances during storage, which is indicated by the results of the detection of amino-ammonia nitrogen (AAN). The AAN content in processed samples on the 15th, 30th and 60th days of storage was 6, 12 and 10 times lower, respectively [73].

Therefore, analysis of the results obtained in the investigation of meat treated with HHP at 800 MPa and higher indicates that the use of this technology allows extending the shelf-life of perishable products such as meats, which presents a huge interest in the field of the development and preservation of food reserves.

Conclusion

Based on the data stated above, it is necessary to emphasize that the application of HHP technology for meat product processing at different parameters has wide prospects for use. For example, raw meat material processing at 100–200 MPa directly before the conventional heat treatment can become one of the possible methods for managing the functional-technological characteristics of the meat system. This treatment can be regarded as compensation for the addition of the reduced amount of sodium chloride and sodium phosphate, as well as for the low fat content in mince in manufacturing the comminuted or gel-type meat products. With that, it will ensure the maintenance of sensory and consumer characteristics of finished products as well as an improvement in product composition regarding healthy nutrition.

On the other hand, the application of HHP in a range of 400 MPa to 800 MPa in meat processing is no less promising for increasing its microbiological safety, resistance to oxidative spoilage and an increase in shelf-life without using food preserving agents.

The considerable promise of these two directions in the HHP application is so evident that the development of new technological processes combining different stages of raw material and food system processing with HHP technology at different parameters is expected.

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AN EFFECT OF THE ANIMAL CONDITION AFTER GAS STUNNING ON QUALITY OF SLAUGHTER PRODUCTS FROM LARGE WHITE PIGS

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Keywords: *CO_2 stunning, physiological conditions of pigs, internal organs, meat quality, pH value, moisture holding capacity*

Abstract
The aim of the research was to establish an effect of gas stunning of pigs at slaughter on the condition of the internal organs and pork functional-technological characteristics. The experiment was carried out in the conditions of the operating enterprise on 37 Large White pigs. When using 94% concentration in a chamber, only 65% of animals were assessed as normally stunned, 30% of animals did not have cardiac activity and were considered dead, 5% of animals retained sensibility. Assessment of the condition of the internal organs, dynamics of pH changes, comparison of moisture holding capacity and color characteristics did not reveal substantial and statistically significant differences between slaughter products from normally stunned animals and animals died during stunning. The authors discuss mortality of animals before the beginning of bleeding as a possible cause of similar results of investigation of the internal organs and meat quality from animals with and without cardiac activity after gas stunning.

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Introduction

Today in Russia, more than 50% of pigs sent to slaughter are subjected to gas stunning. This technological operation affects the physiological conditions of animals and can significantly contribute to formation of slaughter product quality, including pork in carcasses and by-products.

From the perspective of animal welfare assurance, the international practice prescribes using high carbon dioxide concentrations (more than 80%) [1]. When pigs are placed in a chamber with a concentration of 80–90% CO_2, the loss of consciousness and sensibility is not instantaneous and occurs during 30 sec. At such a high CO_2 concentration, the duration of the anesthetic effect and unconsciousness of animals increases, and as a consequence, the time interval for performing other slaughter manipulations can be increased.

Unconsciousness is a state of unawareness (loss of consciousness), in which temporal or permanent damage of brain function occurs, when an animal cannot respond to normal stimuli including pain. An animal is considered insensible when it does not show any reflexes or reactions to stimuli such as sound, odor, light or physical contact [1,2]. The use of CO_2 stunning implies the unconscious condition with retention of cardiac activity in an animal from the moment of its unloading from a stunning chamber until the end of bleeding.

However, at concentrations higher than 30%, this gas causes aversion in animals, irritation of the mucous membranes, painful sensations, lung hyperventilation and gasping before loss of consciousness [3]. In several cases, these sensations can provoke the spontaneous development of stress in pigs up to cessation of cardiac activity. From the perspective of the animal slaughter technology for producing meat and by-products, cessation of cardiac activity can lead to incomplete bleeding of a carcass and consequently to a decrease in quality of slaughter products [4,5]. In the context of physiology, death is a state of an animal, when respiration and blood circulation have ceased. In the context of using stunning at slaughter, the main clinical signs of death are absence of breathing, absence of pulse and pupil dilation [2].

Despite the wide practice of using gas stunning in the world and international documents stipulating regular monitoring of the process and animal condition [1], any recommendations on the control of the number of animals died during stunning are absent. Nevertheless, it is acknowledged that meeting criteria such as absence of reactions in animals can be linked with complete cessation of cardiac activity [2].

A result of gas stunning is influenced by many factors including individual characteristics of animals determined by a breed and keeping conditions. Modern techniques and technologies of gas stunning used in Russian enterprises are foreign developments. In this connection, scientific research aimed at studying an effect of gas stunning on quality of slaughter products from domestic production are of high topicality, especially regarding breeds such as Large White that are traditionally raised in our country. Although recently a reduction in the number of pig breeds has been observed in Russia, Large White breed remains to be the main (52%) part of the stock of breeding animals raised on an industrial scale [6].

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The aim of this study was detection of possible deviations in quality of slaughter products depending on the physiological condition of Large White pigs after gas stunning.

Materials and methods

The experiment was carried out on Large White pigs (n = 37) at the age of 160 days, raised in a selection center and transported to the place of the experiment in one road vehicle. The distance of transportation was 6.3 km. After 3 hours of resting in the enterprise, pigs were sent to slaughter.

Pigs were exposed to carbon dioxide in a chamber Banss (Germany) with five pigs per chamber. The carbon dioxide concentration was 90%, time of exposure was 115 sec. After unloading pigs from a chamber, the result of animal stunning was determined, recording one of the following variants of the physiological condition: norm, retention of sensibility (insufficient effect of stunning), animal death.

A stunning result was considered normal if pigs retained cardiac activity and at the same time did not have signs of sensibility, which corresponds to the requirements of the Technical Regulation TR CU034/2013 «On meat and meat product safety» and international norms. The heart rate (HR) was recorded using a veterinary pulse oximeter DIXION Storm 5000 vet (Dixion, China), attaching a sensor to the ear of an animal after the exit from a gas stunning chamber.

A result of stunning was considered insufficient if animals showed the sings of sensibility (including attempts to stand up and/or raise head, ear movements, vocalization, rhythmic breathing, eye movement and pupil reaction, response to prick).

The animal death was recorded when cardiac activity was absent. A conclusion about absence of cardiac activity was made after triplicate pulse measurements using a pulse oximeter. With that, each time a sensor was fixed at a different point of an ear to exclude a measurement error due to surface contamination of skin or absence of a large vessel in the area of sensor fixation.

At the following stage of the experiment, the internal organs (heart, lung, liver) were examined by assessing a degree of blood filling of tissues and vessels, presence of blood clots in the cardiac chambers and for lungs, additionally, hemoaspiration.

A degree of blood filling of the internal organs was classified as follows:

— normal or insignificant: when pressing a tissue cut, blood does not appear, blood filling is visually observed on a tissue cut only in the individual areas;
— medium: blood filling is visually detected on a tissue cut; when pressing a tissue cut, blood appears; blood vessels are filled with blood;
— high: significant blood filling is visually detected on a tissue cut; blood runs out without pressing; blood vessels are significantly filled with blood.

After primary processing, carcasses were chilled to a temperature not higher than 4 °C for 24 hours using the classical chilling technology.

Pork pH and temperature were measured in the hot and chilled state 45 min. and 24 hours after slaughter in *m. longissimus dorsi* between the 5th and 6th thoracic vertebrae in a half-carcass using a pH-meter Testo 205 (Testo, Germany). During measurements, an electrode was placed into muscle tissue at a depth of not less than 3 cm. Arithmetic mean of three single measurements was taken as the final result, divergence between ultimate values of three measurement results was not higher than 0.15 pH units.

Samples of *m. L. dorsi* (about 300 g) were taken from chilled half-carcasses 24 hours after slaughter for further laboratory analyses.

Drip losses were determined as weight reduction in pork samples during storage in a hermetically sealed container at a temperature of 8 °C for 24 hours. The moisture holding capacity was measured by the Grau and Hamm method in modification of VNIIMP. Arithmetic mean of three single measurements was taken as the final result.

The color of muscle tissue was determined in the CIELab color space using a spectrophotometer Konica Minolta CM-2300d. The color was measured at three points on the sample surface. Arithmetic mean of three single measurements was taken as the final result.

Statistical processing of the experimental data was carried out using Microsoft Excel 2010 and Statistica 10.0. Significance of differences between means that satisfied the conditions of normal distribution and equality of dispersion was assessed by one-way analysis of variance (ANOVA). The results were presented as M_{mean} (weighted average of measured values) ± s (standard error of the mean).

Results and discussion

Hartung J. et al. [7] reported that the use of gas stunning at 90% CO_2 led to death of most animals before the beginning of bleeding. In our experiment, assessment of the condition of Large White pigs after gas stunning (Table 1) showed that 24 animals (65% of total number) were stunned normally; that is, they did not demonstrated the signs of sensibility, but retained cardiac activity. In 11 pigs (30%), the death was diagnosed as a result of absence of heart beat recorded by a pulse oximeter. In two animals (5%), retention of sensibility was observed indicating an insufficient effect of CO_2 stunning. Nevertheless, our results showed quite a high proportion of dead animals, which consequently can create problems regarding meat hygiene [7].

There is another approach to studying the physiological conditions of animals during gas stunning, which consists in registration and recording electroencephalograms (EEG) using special devices attached to animals. Special sensors register changes in brain activity including the onset of unconsciousness or death. However, implementation of this approach in the production conditions faces significant difficulties. It is necessary to shave animal heads beforehand in the areas of electrode fixation (the forehead part), attach belts with devices and wires to electrodes to animals and to train animals to wear them for several days [7,8]. Our approach

Table 1. Assessment of the animal condition after gas stunning

No. of animal	Assessment of the animal condition after stunning		
	Presence (+) / absence (–) of heart beat	Presence (+) / absence (–) of sensibility signs	General conclusion about animal condition
1	+	–	Norm
2	–	–	Death
3	+	–	Norm
4	–	–	Death
5	+	–	Norm
6	+	–	Norm
7	+	–	Norm
8	+	–	Norm
9	–	–	Death
10	–	–	Death
11	+	–	Norm
12	+	–	Norm
13	+	–	Norm
14	+	–	Norm
15	+	–	Norm
16	+	–	Norm
17	+	–	Norm
18	+	–	Norm
19	–	–	Death
20	–	–	Death
21	–	–	Death
22	+	–	Norm
23	+	–	Norm
24	+	–	Norm
25	–	–	Death
26	–	–	Death
27	+	–	Norm
28	–	–	Death
29	+	–	Norm
30	+	–	Norm
31	–	–	Death
32	+	–	Norm
33	+	–	Norm
34	+	–	Norm
35	+	–	Norm
36	+	+	Retention of sensibility
37	+	+	Retention of sensibility

based on measuring heart rate probably is less informative but simpler for implementation in production conditions. It is also necessary to note that in the experiment with EEG registration, which was carried out at 95% CO_2, all animals were dead over 5 min. due to irreversibility of unconsciousness [8].

Based on the assessment results regarding the physiological conditions of 37 animals (Table 1), ten of them were selected by the method of random sampling including five pigs from normally stunned animals and five from dead animals. The results of the examination of the internal organs obtained as a result of evisceration of the carcasses from these animals are presented in Table 2.

As can be seen from Table 2, the internal organs from carcasses of normally stunned animals were filled with blood to the same (and even to a slightly higher) degree as the organs from the carcasses of the dead animals. This result was somewhat unexpected, as it was assumed that bleeding was more complete when cardiac activity was retained. However, the obtained data still give little grounds to state that cardiac arrest at stunning does not affect a degree of carcass bleeding.

The following results were obtained for ten carcasses selected for assessing the condition of the internal organs: 70% of hearts and 70% of lungs were characterized by the high degree of blood filling. With that, the high degree of blood filling was noted only for 30% of the liver samples. These results allow suggesting that cardiac arrest in animals could occur after pulse measurement but before the beginning of bleeding.

The hot carcass weight, pH value after 45 min (pH_1) and 24 hours (pH_{24}) were measured for each of 37 carcasses (Table 3). By the results of pH_1 measurement, PSE pork carcasses were selected, in which a pH value 45 min. after slaughter was lower than 6.0, and pH24 was lower than 5.5. Carcasses that had only one low pH value (pH1 or pH_{24}) were classified as meat with signs of PSE.

As can be seen from Table 3, pork from animals without cardiac activity and pork from normally stunned animals practically did not differ by the mean values of pH after 45 min. and 24 hours. However, carcasses of dead animals demonstrated higher inclination to the development of the PSE defect (36% carcasses versus 25%).

Table 2. Assessment of the condition of the internal organs

No. of animal	Assessment of the condition of the internal organs:		
	heart	lung	liver
Carcasses from normally stunned animals			
1	Blood filling +	Blood filling +	Blood filling +
3	Blood filling +++, blood clots in cardiac	Blood filling +++, hemoaspiration	Blood filling +++
5	Blood filling +++, blood clots in cardiac	Blood filling +++, hemoaspiration	Blood filling ++
18	Blood filling +++, blood clots in cardiac	Blood filling +++, hemoaspiration	Blood filling +
27	Blood filling +++, blood clots in cardiac	Blood filling +++, hemoaspiration	Blood filling +++
Carcasses of animals, in which death was observed after gas stunning			
2	Blood filling +++, blood clots in cardiac	Blood filling ++, hemoaspiration	Blood filling +
4	Blood filling ++	Blood filling +, hemoaspiration	Blood filling +
20	Blood filling +++, blood clots in cardiac	Blood filling +++, hemoaspiration	Blood filling +
21	Blood filling +	Blood filling +++, hemoaspiration	Blood filling +
25	Blood filling +++, blood clots in cardiac	Blood filling +++, hemoaspiration	Blood filling +++

Designations: Blood filling +: norm or insignificant degree of blood filling of tissues of the internal organ;
Blood filling ++: medium degree of blood filling of tissues of the internal organ;
Blood filling +++: medium degree of blood filling of tissues of the internal organ.

Table 3. Hot carcass weight and pork pH value

No of animal	Hot carcass weight	pH value		Quality group
		pH_1	pH_{24}	
Carcasses from normally stunned animals				
1	69.8	6.10	5.62	NOR
3	60.0	5.75	5.45	PSE
5	66.4	6.16	5.43	with signs of PSE
6	66.8	6.11	5.49	with signs of PSE
7	70.2	6.12	5.53	NOR
8	57.2	5.68	5.57	PSE
11	58.8	5.87	5.54	PSE
12	68.8	6.36	5.55	NOR
13	61.8	5.97	5.43	PSE
14	61.6	6.03	5.54	NOR
15	68.6	6.07	5.90	NOR
16	60.6	6.82	5.53	NOR
17	56.6	6.02	5.74	NOR
18	77.4	6.04	5.52	NOR
22	75.8	6.01	5.57	NOR
23	62,2	6,37	5.69	NOR
24	56.0	6.49	5,59	NOR
27	63.2	6.00	5.60	NOR
29	63.8	6.24	5.55	NOR
30	69.2	6.45	5.52	NOR
32	56.6	6.15	5.64	NOR
33	77.2	6.28	5.64	NOR
34	63.8	6.32	5.65	NOR
35	59.8	6.12	5.62	NOR
Mean value	64.68±6.41	6.15±0.25	5.58±0.10	
Carcasses of animals, in which death was observed after gas stunning				
2	63.2	6.34	5.52	NOR
4	57.2	5.92	5.45	PSE
9	77.6	5.31	5.49	PSE
10	53.4	5.42	5.53	PSE
19	66.2	6.15	5.52	NOR
20	74.2	6.07	5.63	NOR
21	74.4	6.30	5.48	with signs of PSE
25	68.8	6.22	5.75	NOR
26	88.4	6.05	5.62	NOR
28	61.0	6.38	5.60	NOR
31	53.2	6.44	5.68	NOR
Mean value	67.05±10.97	6.05±0.38	5.57±0.09	

There is an opinion regarding pork that CO_2-stunning does not provoke glycogen breakdown and consequently pH drop [9]. Other studies allowed making a opposite conclusions. For instance, it was shown by the example of turkey that CO_2- stunning can lead to meat with lower pH compared to electrical stunning [10].

Mota-Rojas D. et al. rightly indicate that when CO_2 is respired, it combines with water with the development of H_2CO_3 (carbonic acid), which generates high concentrations of H+ ions resulting in the state of acidosis on the cellular level. Inhalation of high CO_2 concentrations can cause distress in animals (a negative form of stress) before they become unconscious. Pigs exposed to CO_2 experience respiratory and metabolic acidosis. The blood level of lactate, which is an indicator of stress, increases. An increase in the lactate level can be linked with stress factors before slaughter (for example, aggressive handling immediately before stunning) and negatively affect pork quality [11].

In our experiment, animal death upon stunning can be explained by their individual characteristics and higher susceptibility to stress. This, in turn, can provoke an appearance of a higher number of PSE carcasses from animals with cessation of cardiac activity.

A result of pig stunning can depend on their weight. In our experiment, there was no possibility to measure the animal live weight and/or weight after stunning; therefore, we compared two animal groups by the hot carcass weight. The mean value of the hot carcass weight from dead animals was slightly higher than those from normally stunned animals (67.05 kg and 64.68 kg, respectively). However, the result of Student's t-test calculation showed that differences of the mean values of the carcass weight were not statistically significant ($t = 0.17$, $p = 0.853$, the critical value of Student's t-test = 2.035 at the significance level $\alpha = 0.05$).

There were only two animals in the experiment that retained sensibility. An average weight of hot carcasses of these animals was 79.5±2.4 kg. A test of the hypothesis about the difference in the carcass weight between these animals and animals that were normally stunned showed that the differences were statistically significant ($t = 2.14$, $p = 0.043$, the critical value of Student's t-test = 2.064, at the significance level $\alpha = 0.05$). However, due to insignificance of sampling ($n = 2$), conclusions about an effect of increased weight of Large White pigs on retention of sensibility upon gas stunning would be premature and require a set of larger volumes of experimental data.

Statistical data analysis did not reveal correlation between the hot carcass weight and pH values.

Functional-technological indicators of meat quality and color characteristics (Table 4) were investigated randomly on the samples from ten carcasses selected in a random manner. Statistical analysis using Student's t-test did not reveal significant differences between pork obtained from normally stunned and dead animals. The mean values of moisture holding capacity (MHC) were 74.75% and 74.71%, drip losses were 6.50% and 6.19%, respectively, for the samples from the carcasses of normally stunned and dead animals. The samples also did not differ by the mean values of moisture mass fraction (74.75% and 74.71%, respectively).

By color characteristics, pork from Large White pigs was characterized by quite high values of lightness (L*) and low values of redness (a*); although when using CO_2-stunning, pork usually has darker color [9]. The differences in color between pork samples from normally stunned and dead animals were also not established.

Table 4. Pork functional-technological indicators and color

No. of animals	Drip losses, %	MHC, %	Moisture, %	Color characteristics		
				L	a	b
Carcasses from normally stunned animals						
1	4.77	55.64	73.29	48.19	3.72	8.34
3	5.95	55.77	74.51	55.17	2.41	8.35
5	5.66	53.06	75.37	55.75	3.94	8.60
18	8.34	54.01	75.4	53.10	4.00	7.57
22	7.80	49.38	75.2	59.05	2.45	8.50
Mean value	6.50±1.51	53.57±2.60	74.75±0.89	54.25±4.01	3.30±0.80	8.27±0.41
Carcasses of animals, in which death was observed after gas stunni						
2	5.50	55.00	74.37	55.20	2.75	8.13
4	5.80	55.96	74.47	53.90	2.86	8.45
19	5.21	50.10	73.42	55.14	2.81	7.84
20	7.27	54.83	75.48	58.23	1.95	7.52
21	7.15	53.48	75.81	56.71	1.80	8.00
Mean value	6.19±0.96	53.87±2.29	74.71±0.95	55.84±1.67	2.43±0.51	7.99±0.34

Conclusion

By the results of the performed work, the further study on an effect of CO_2-stunning on quality of pork from Russian pig breeds is considered an important task, including the aspects of animal welfare monitoring in national enterprises. The results of the research show that regarding Large White pigs, up to 5% of animals (especially larger animals) can retain signs of sensibility at 90% gas concentration and exposure duration of 115 sec. Five percent of animals is a good result. For example, studies carried out in different German enterprises demonstrated that 6.2–17.1% of pigs retained corneal reflex [12].

Detection of presence/ absence of cardiac activity using a pulse oximeter is a convenient method for controlling an animal condition upon unloading from a stunning chamber, which is feasible for using in the work of a production line. Using a pulse oximeter, cessation of cardiac activity was recorded in 30% of animals. However, cardiac arrest practically did not influence either a degree of the internal organ bleeding or meat quality including meat color characteristics. Obviously, it is necessary to control presence/ absence of the pulse and signs of sensibility immediately before bleeding to obtain more objective picture of physiological conditions of animals.

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THE ELECTROHYDRAULIC METHOD FOR MEAT TENDERIZATION AND CURING

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Keywords: *electro-hydraulic equipment, tenderization, whole-piece meat semi-finished product, curing, intensification*

Abstract
The paper examines the problem of meat raw material curing in production of whole-piece meat products. The intensification methods for the process of penetration and distribution of curing ingredients throughout a product are described. Design of the equipment for meat tenderization URM-1 and URMP-1 is proposed, which ensure electrohydraulic tenderization of the structure of whole-piece (1500–2000 g) and portioned (80–120 g) meat semi-finished products and accelerate a process of brine distribution, which will allow reducing product strength characteristics by 51–53 kg/cm, reducing raw material losses, increasing labor productivity by 8–11%, shortening the duration of the technological process and reducing energy expenditure upon heat treatment by 18–20%. As a result of the experimental investigations, it was established that an electrohydraulic impact (frequency of pulses ν = 0.5–1.0 pulse/sec., number of pulses from 150 to 200) can be used for tenderization of muscle connective tissue and tendons both of chilled (core temperature of 0 °C to 4 °C) and subfrozen (–2 °C to 3 °C) meat.

Introduction

Due to their palatability and nutritional quality, whole-piece semi-finished products are in great demand among the population. Prime cost reduction and assurance of required quality are important requirements for producers and consumers of such products. The development of new technologies will allow increasing output and broadening an assortment of semi-finished products.

Taking this into consideration, the authors developed a technology of meat semi-finished products in pieces treated in brine by the electrohydraulic method. Raw material curing is one of the most complex and durable technological processes when producing whole-piece products. During curing, mass exchange takes place in the system brine-meat between curing ingredients and meat components. To accelerate mass transfer and improve functional-technological properties of meat raw materials, different intensification methods for the curing process are used: biochemical, physical, mechanical and acoustic [1,2,3,4,5]. Analysis of publications on this question also indicates the use of electrophysical and physico-mechanical methods of meat treatment [6,7,8]. Methods based on pulsed energy impacts can be promising for accelerating the process of meat raw material curing [9,10].

The aim of the work was to create equipment for electrohydraulic tenderization and curing of whole-piece products and develop technological parameters of processing.

Materials and methods

Meat from pork, beef and mutton shoulder and reindeer loin was used as objects of research. To process portioned pieces with a weight of 0.08–0.12 kg, we used the URM-0.1 unit (Figure 1) [11,12,13,14,15,16,17,18,19], which works as follows. Samples are placed into the body 7 of the unit; the electrodes 9 located at its bottom penetrate the tissue structure at a depth of 3–5 mm. Then, the cover 3 is closed up to the tight contact with the skirt 6, which allows electrodes 4 to penetrate the structure of a semi-finished product from the top to the depth of 3–5 mm. Using the switch 2, electric energy is supplied and the source of pulse voltage is made operational. Discharge pulse comes to the electrodes by the pulse buses 10. A spark occurs inside a semi-finished product and discharge pulsed treatment takes place.

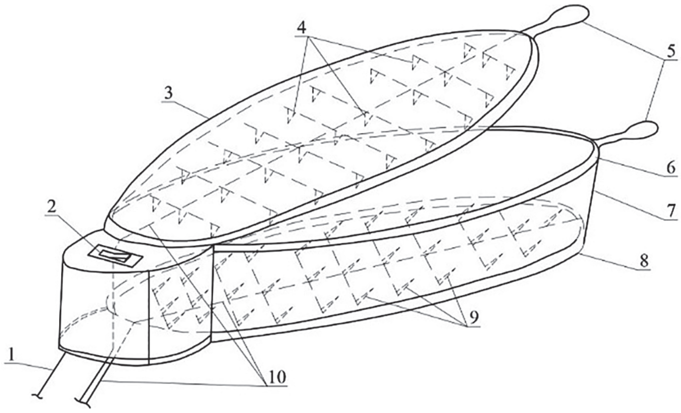

Figure 1. A scheme of the unit for meat tenderization URM-1: 1 — grounding; 2 — switch; 3 — cover; 4, 9 — electrodes; 5 — handle; 6 — skirt; 7 — body; 8 — base; 10 — pulse buses

The URMP-1 unit (Figure 2) was used to process large pieces (1–1.5 kg). Semi-finished products were placed on the grid 3, which was put into the container 1 and covered with brine. High voltage pulses that created discharges in brine were applied to discharge contacts, which resulted in

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their distribution from the cavity to the grid and a product, where they lost their energy. This ensured appearance of a mechanical impact, which tenderized tissue and significantly accelerated penetration and distribution of brine throughout a meat piece.

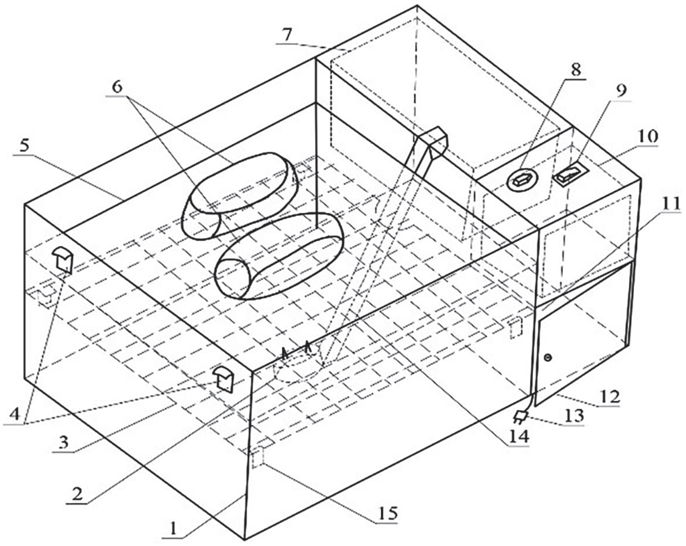

Figure 2. A scheme of the unit URMP-1 for tenderization of whole-piece meat semi-finished products: 1 — container; 2 — discharge contacts; 3 — grid; 4 — hinges; 5 — brine; 6 — whole-piece meat semi-finished products; 7 — high-voltage transformer; 8 — timer; 9 — switch; 10 — surge current generator; 11 — storage place for wires; 12 — door; 13 — electric wires; 14 — conductor; 15 — fastener

Technical characteristics of the URM-1 unit for meat electrohydraulic tenderization and curing: voltage 220–230 V, current 1 A, power 450 W, current frequency 50 Hz. Technical characteristics of the URMP-1 unit: VWCh = 0.1 m^3, voltage 15 kV, current 1 A, power 1 kW, current frequency 50 Hz.

URM-1 is used for portioned semi-finished products with treatment duration of 5–7 sec. and discharge interval of 20–30 μs.

For a large piece, URMP-1 is used with treatment duration of 2–3 min., discharge interval 30–50 μs, spark speed 4300 m/s.

Results and discussion

The proposed method for tenderization of intramuscular and intermuscular connective tissues and muscle fibers uses the impact of electrical discharges (an electrohydraulic impact — EHI), that occur in the inner layers of a product with a frequency of f = 50 Hz and duration of 20–30 μs. This facilitates sharp expansion of liquid in a semi-finished product and development of a gas channel that ensures the first hydraulic impact. The channel is closed under an effect of elasticity of water and meat muscles, then the second EHI takes placed and so on. With that, the pressure in the discharge area and at a distance of 1.5–2 cm reaches a few tens of kgf/cm^2 and more.

Each following electrical discharge increases tissue destruction. With that, there is an increase in a number of free surfaces (concentrates of energy), where breaking strain and rupture of the tissue structure occur upon reflection of falling shockwaves. At constant generation of a series of electrical discharges, each preceding discharge creates additional free planes as a result of tissue rupture creating new slots. As a result, a degree of using the energy of voltage waves increases more and more, and resistance of connective tissue inclusions decreases. Muscles of the internal structure of a semi-finished product are dislocated to a distance of 3–8 mm. Strength characteristics of a meat semi-finished products are reduced.

It is necessary to note that released energy from EHI is selective to the places with increased strength of meat tissues, which ensures meat tenderization [21,22,23,24,25,26, 27,28,29].

The developed method realizes the effect of the electrohydraulic impact that ensure accelerated penetration and distribution of curing ingredients throughout a product due to loosening the structure of meat tissues [17,18,19, 29,30,31,32,33,34].

As publications [21,22,23] show, the proposed method reduces drip losses, increases assimilability of meat products due to disruption of protein bonds and extension of intercellular space, improves structural-mechanical properties of products with a simultaneous increase in product stability to the development of microorganisms (in raw materials and finished products).

Figure 3 presents the parameters and technological scheme of producing cured meat semi-finished products using URM-1 and URMP-1.

The technological process includes the following operations: preparation of initial meat raw materials and brine. Thawed or chilled half-carcasses are trimmed, divided into cuts and deboned; large piece semi-finished products and portioned semi-finished products are separated. When processing bone-in products, a cut is divided into pieces with a weight of 1–1.5 kg.

The proposed technology includes three stages: electrohydraulic action on the meat structure; separation of whole-piece semi-finished products into portions and storage.

Figure 4 presents the microstructure of a meat semi-finished product after processing using EHI (200 pulses during 100–120 sec.) with penetration of electrodes into a product to a depth of 3–5 mm. As a result of the action of electrical discharges, the connective tissue layers and muscle structure are destructed. Due to brine penetration into meat, muscle fibers swell and their volume increases.

The results of the experimental study on the mechanical processing of semi-finished products from pork, beef and mutton using the electrohydraulic impact are presented in Table 1 and Table 2.

The obtained data indicate that the electrohydraulic impact on meat raw materials increases volumes of samples compared to initial by 47–61%, the thickness of connective tissue layers increases by 3.3–7.5 times and the weight of semi-finished products increases by 5–10%.

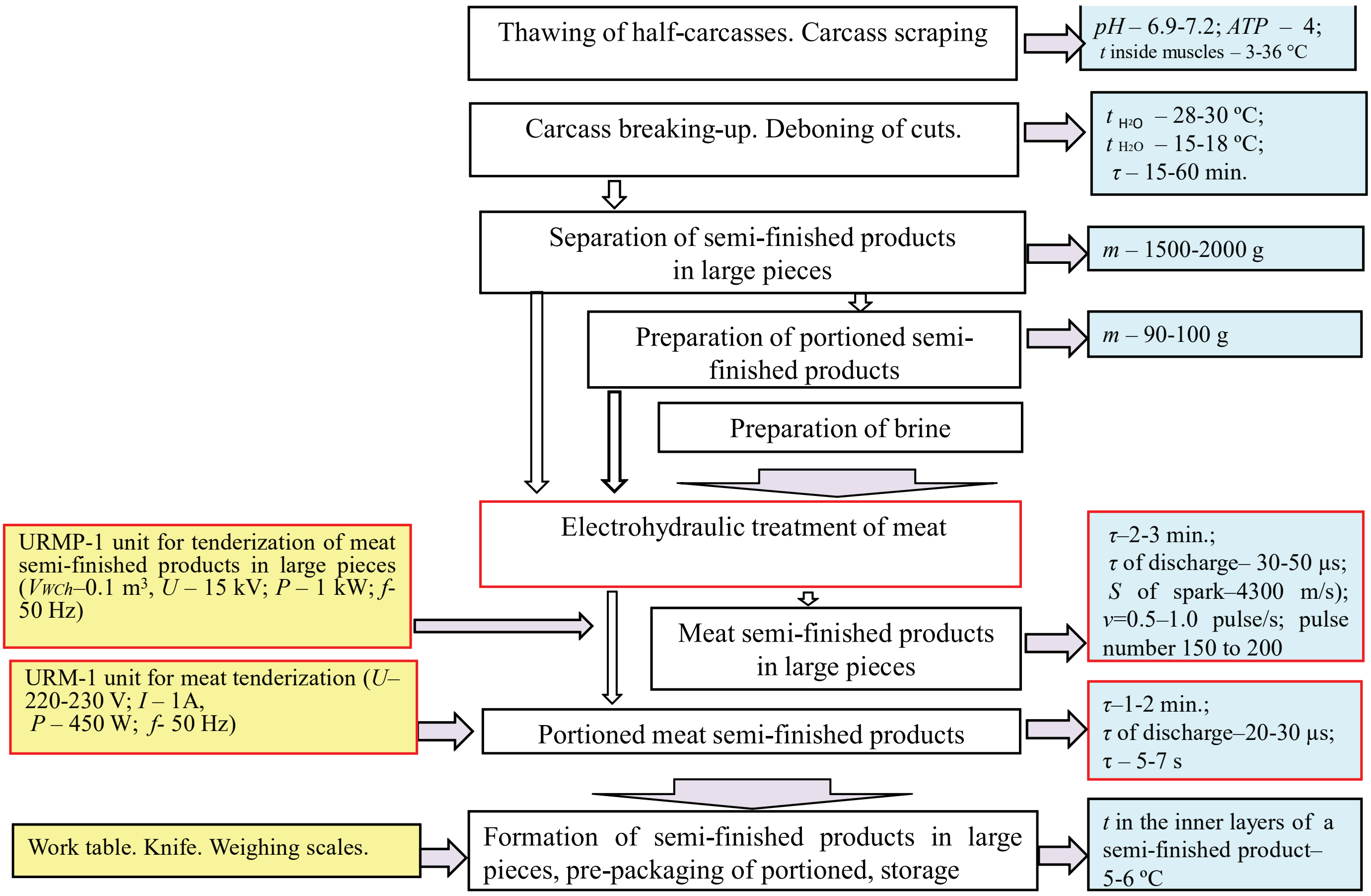

Figure 3. Technological scheme for production of cured meat semi-finished products

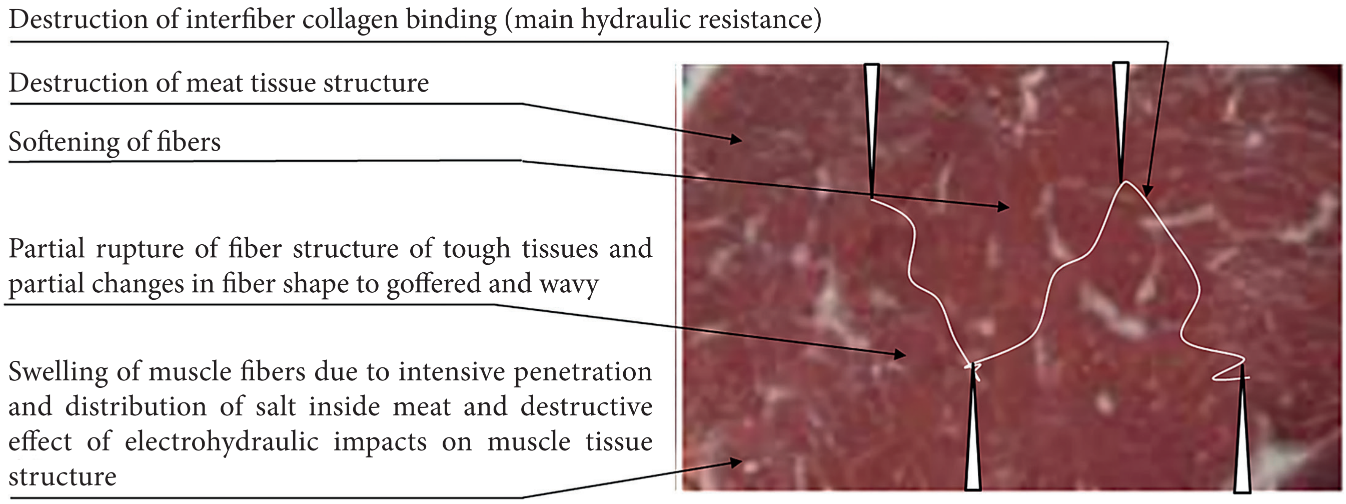

Figure 4. Microstructure of the cured semi-finished product after mechanical action of the electrohydraulic impact

Table 1. Changes in the product characteristics as a result of the electrohydraulic impact

Indicators	Meat type		
	Beef, 1st category	Pork, 1st category	Mutton, 1st category
Thickness of meat semi-finished product, mm initial raw material after EHI	17–18 25–28	16–18 26–29	17–19 24–25
Thickness of softened collagen layers, mm initial raw material after EHI	About 0.7 mm 2–4 mm	About 0.8 mm 4–6 mm	About 0,6 mm 2–4 mm
Weight of semi-finished products, g initial raw material after EHI	101±2 106–109	102±2 109–112	104±2 109–110
Increase in portion weight,%	5–8	7–10	5–7
Color of semi-finished products	Insignificantly decolorized		

Table 2. Comparative characteristics of the nutritional value of portioned semi-finished products processed by the traditional and proposed methods

Indicators	Content in portion			
	Initial raw materials	Traditional method	Tenderization in URM-1	Magnification
Caloricity, kcal	168	96	163	+ 67
Proteins, g	20	12	19.4	+7.4
Fats, g	9.8	6	9.7	+3/7
Water, g	69.2	45.2	68.8	+ 23.6

Table 2 presents the nutritional value of experimental semi-finished products and those produced by the existing technology.

After corresponding treatment, meat semi-finished products can be used both for storage (freezing) and for cooking (heat treatment).

Experimental studies on electrohydraulic tenderization and simultaneous curing of portioned meat semi-finished products from the inner part of reindeer loin with a weight of 80–120 g and whole-piece meat semi-finished products with a weight of 1000–1500 g that were not subjected to trimming are presented in Figure 5.

The obtained data show that as a result of reindeer loin treatment using EHI, shear force decreased by 27–32% and breaking force by 18–23%.

Conclusion

Electrohydraulic treatment of meat using the URM-1 unit or URMP-1 unit allows decreasing product losses, increasing tenderness and reducing curing process duration compared to curing in the mechanical equipment.

For practical implementation, it is recommended to use the proposed equipment to process semi-finished products in large pieces with a weight of 1–1.5 kg and portioned semi-finished products with a weight of 0.08–0.120 kg. This will allow reducing product strength characteristics by 51–53 kg/cm, decreasing raw material losses, increasing labor productivity by 8–11%, shortening technological process duration and decreasing energy expenditure upon heat treatment by 18–20%.

The electrohydraulic impact (frequency v = 0.5–1.0 pulse/sec., number of pulses from 150 to 200) can be used for muscle and connective tissue tenderization of both chilled (core temperature of 0 °C to 4 °C), and sub-frozen (–2 °C to 3 °C) meat.

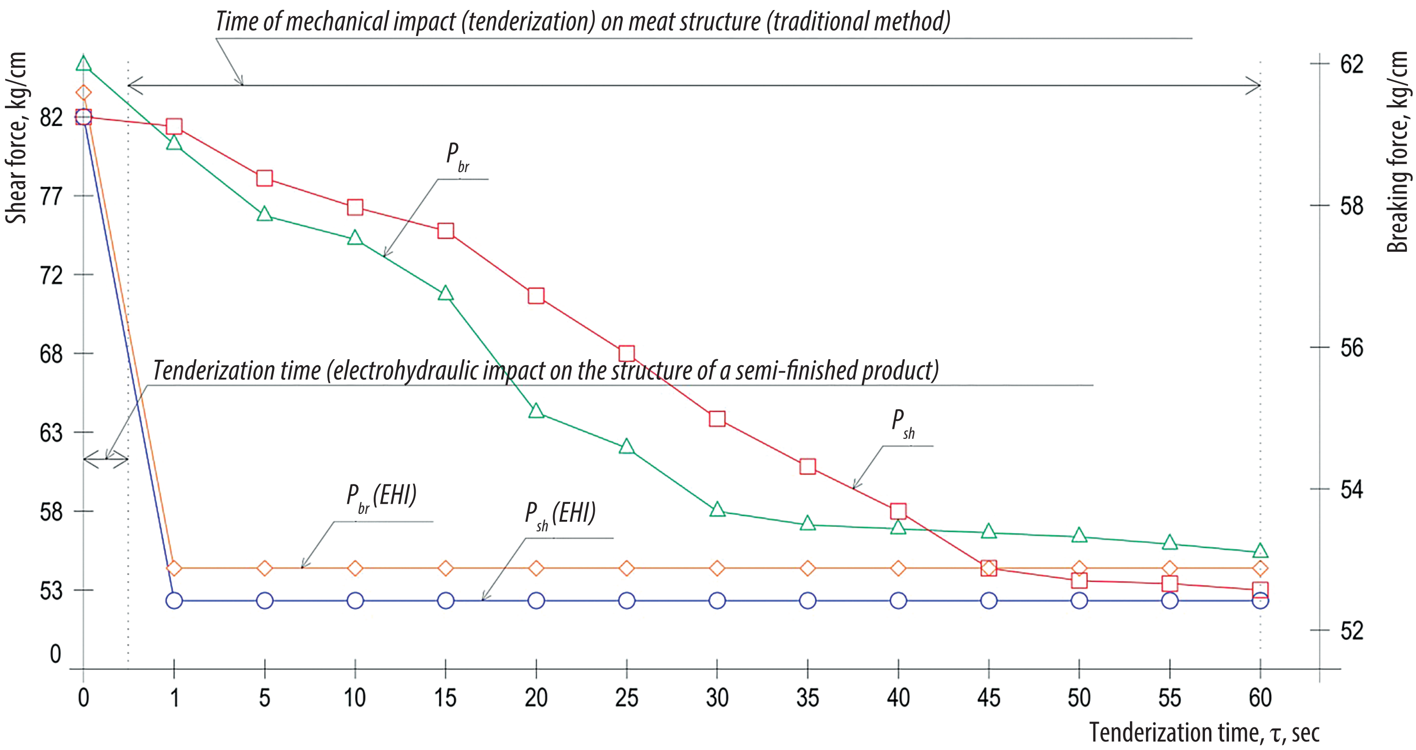

Figure 5. An effect of electrohydraulic treatment on strength properties of muscle and connective tissues of reindeer loin

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