

To The Issue of Meat Safety in Kazakhstan

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Kazakhstan has become the only former Soviet republic which has developed national halal products standard, in 2006 Kazakhstan received the international certificate proving high quality of these products and headed the list (having received the Grand Prix of the international exhibition of products produced in accordance with the Sharia Islamic Law)¹. Considering the mentality of our eastern country, and tendency to maintain stable relations with the foreign Islamic countries, introduction of this quality standard opens new page in struggle for quality. Halal products have a variety of advantages compared with the products produced using traditional technologies and their taste is superior to non-halal products, that is explained by the following features: the grace is said, then carotid artery is slashed that provides complete removal of blood from carcass. Due to this the meat has sophisticated flavor, animals used for production of halal meat are bred on environmentally safe feed, and any hormonal additives are excluded. Influencing of cortisol and adrenalin hormones to the meat quality was studied by several scientists², also impact of pre slaughter stressors on ruminant carcass and meat quality was studied by D.M. Ferguson and R.D. Warner³.

Key words: Halal products, cortisol, blood, mutton meat, beef, state standard, antibiotics, microbiological safety.

Over two years ago Kazakhstan launched development, improvement and methods of introduction of the Malaysian halal standard MS 1500:2004, which fully complies with the Islamic canons and requirements. Currently, halal standard products, in particular, meat products are already widely popular. And this is not surprising: because halal means food products produced in accordance with the Muslim traditions, which are consumed by people of any nationalities and confessions. The Hadji Association of Kazakhstan even established the special technical committee for issues of an international standard MS 1500 – Halal Food.

Moreover, “halal” labeling is perceived to be additional quality guarantee and product safety, the mark meaning that it contains no harmful chemicals.

Animals killing is performed in accordance with Islamic norms. The livestock the day before killing shall be healthy. It is killed through intersection of cervical arteries, in a single step, without delay and interruption, with the name of Allah on the lips simultaneously with intersection of main cervical arteries and gullet, better closer to the head. Carotid artery of every animal is intersected so as blood may completely discharge, that cannot be achieved in existing ways of killing: nervous breakdown with electrical shock, brain affection using mechanical attack, anesthetizing using carbon dioxide and any other chemicals, coagulated blood of the animal stays in the meat⁴.

In this case cortisol, stress hormones start

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discharging into the animal blood, which further on start entering the human body harmfully affecting the human health. This also immobilizes it during killing, that in turn results in significant or complete disturbance of blood extraction. It is of crucial importance that during killing of one animal, the others do not see this so as not to be scared and avoid stress and stress hormones release.

In accordance with religious norms it is extremely important that blood flows from killed animal under influence of “natural convulsion”.

Basic principles of the Islamic “Halal” mean humane treatment of animals and birds before killing, an animal should be alive and healthy, careful avascularization of animals with its heart functioning.

In II century of the last millenium Ibn Sina (Avicenna) was the first to demonstrate fear impact by the example of a lamb placed in cage located in close proximity to wolf’s cage so that animals were within visual range of each other. The lamb relatively quickly died from developed fear, depression, disorders of its heart-vascular system and breathing functioning system.

Fear, anxious expectancy and behavior conflicts result in disorders of high nervous action and, by definition of E.P. Pavlov (1924), to “tilt” of excitative and inhibitory processes in the central nervous systems (CNS) [5]. The reflectory theory was enriched by studies of A.A. Orbeli (1923) of adaptive and trophic function of sympathetic nervous system and À. D. Speransky (1935) about nervous trophism in disease and recovery processes⁶. W. Canon (1929, 1932) stated homeostatic principle having showed that unity and stability of internal environment is supported by a series of comprehensive and multiform processes, in which practically universal role belongs to sympathy- adrenal system. The concept of stress and general adaptation syndrome, stated by Í. Selye (1936, 1956) had been developed in works of L. Levy (1980), based on functional systems theory⁷.

Cortisol in human organism is produced by adrenal cortex with direct involvement of ACTH hormone produced by hypophysis. It is one of the most important elements in body response to stress situations. Deviations in this hormone levels may cause various diseases. Cortisol - a hormone produced by renal gland, was called “The Stress

Hormone”. As a rule, it presents in our system, with higher morning and evening levels. Its functions include adjustment of blood pressure, glucose, insulin, blood sugar balance, inflammatory responses and immune functions⁸.

Animal experiments showed that “stress hormone” cortisol is a cause of mental disorders in diabetes. Doctor Mark Matson, Princeton University, explained that one of target organs of diabetes is brain cord. For this reason, disorder of some cognitive (perceptive) functions occurs⁹.

In accordance with Matson, identification of cortisol memory impact is of great scientific interest. The investigator supposes that hormone’s impact mechanisms on neuron links deserve high attention.

As to practice, the scientists will have to find out if cortisol blocking agents may have positive effect on diabetics. Determination of animal cortisol blood levels is clinically significant during determination of pathogenesis of such changes in the organism like anemia, heart functioning reduction, gastroenteric upset, muscle atrophy, etc., which development may occur in case of insufficient functioning of adrenal cortex, and at investigation of disorders of water-salt metabolism, protein metabolism and carbohydrate metabolism at clinical examination of animals¹⁰.

Basic level of cortisol is subject to heavy fluctuations depending on physiological state. Cortisol level (ng/ml) in Black-and White cows 31-60 before calving is within the range of 56.6 ± 8.8 , 21-30 before calving - 70 ± 5.1 , 1-10 before calving – 80.9 ± 12 , in the day of calving- 120.3 ± 12 , 1-10 after calving - 35.1 ± 4.4 , 11-30 days later – 43.2 ± 2.3 . Cortisol blood concentrations in muttons vary from 100 to 350 nm/l, ACTH - from 70-700 ng/ml¹¹.

Recent study facilitated establishing that vitamin Ñ also contributes into reduction of physical and psychological manifestations of stress. People with high levels of vitamin C, being in psychologically acute situation, show no reactions attributable to stress. Moreover, they decompress faster than people with low blood concentrations on vitamin C.

In animal experiments, vitamin C applied in rats under stress, both prevented expected increase in cortisol level, and avoided occurrence in animals of other known evidences of psycho-emotional stress, including reduced body weight.

In animals which did not receive vitamin C stress hormone levels were three times higher.

It was proved, that cortisol does not mobilize main functional cell proteins, like contractile muscle proteins or nervous tissue proteins, while nearly all other proteins are not released. This preferable use of labile proteins under influence of cortisol meets demand of cells in amino acids for synthesis of life topical substances.

For the purpose of systematic national control over antibiotics pollution of live stock products, the microbiological methods are most widely used both in scientific studies, and in practical institutions, allowing determination of minimum concentrations of antibiotics in study material¹². They are based on immediate biological effect of antibiotics on sensitive microbial strains, so they are most specific and unprejudiced. Antibiotics contents are determined using microbiological diffusion method in agar by value of growth inhibition of the following test cultures placed in nutrient mediums:

- a) For tetracycline antibiotics - *Bac. cereus* ATCC 11778 (sensitivity – 0.01 U/g/ml);
- b) For grizin- *Bac. subtilis* ATCC 6633 (sensitivity 0.5 U/g/ml);
- c) For zynbacitracin - *M. flavus* ATCC 10240 (sensitivity 0.02 U/g/ml).

During slaughter, primary processing of carcasses microbes come with the skins of animals, from the gut, with slaughter and processing equipment, from the surface and through the lymph, blood vessels, tendons and bones along penetrate the meat carcasses. Analysis of the processes of primary meat processing showed that the main hazard at slaughter and primary carcasses processing is the development of microorganisms, so the excess of microbiological indicators due to residual internal organs during evisceration¹³. Microorganisms which known as a biological hazard are: *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella* spp, *Staphylococcus aureus*, *Yersinia enterocolitica*¹⁴.

MATERIALS AND METHODS

The study was carried out in the KJIC (Kazakh-Japanese Innovation Centre) in the

Kazakh National Agrarian University in Kazakhstan.

The study objective was to investigate blood cortisol levels and fixing of cortisol hormone in blood of animals before and after killing.

Cortisol concentrations were determined using immunoenzymatic assay in three muttons of Yedilbayevskays breed in Aydarly village of Zhambyl district, Almaty region, on enzyme immunoassay analyzer using set for cortisol determination “Vector Best” by workers of research diagnostic laboratory of Kazakhstan-Japan Innovation Centre. Test specimen were twice repeated in 14 wells for standards and reference serums. They were placed into relevant wells in duplicates 25 mcl of each standard and reference serum. In other wells they placed 25 mcl of study serum specimen. 100 mcl of conjugate solution were placed into each well. After mixing of plat content was incubated within 60 minutes at 37 °Ñ. After incubation, well contents were suctioned and wells were washed 5 times with 250 mcl of wash solution. 100 mcl of tetramethylbenzidine (TMB) substrate solution was brought into wells within 2-3 minutes. 10-20 minutes of incubation under darkroom conditions at 18-25°Ñ depending on degree of development of blue coloration. 100 mcl of stop solution were placed into all wells, thereat well contents were coloured violent yellow. Optical density was determined in wells using photometer at wave length 450 nm. Plat wells solutions optical density was determined within 15 minutes after adding stop solution. Calibration graph was constructed in semilogarithmic coordinates. Cortisol content in test specimen was determined by calibration graph.

Meat and meat products sampling for determination of antibiotics – maximum 50 g of each sample is performed in accordance with GOST 7269-79. For determination of antibiotics contents they used 6 red meat samples - mutton at temperature of 23°C and humidity 60%.

Portions of 10.0 +/- 0.1 g of soft tissue cut from center portion of a sample were disintegrated using cutting tool with following grinding in pounder with quartz sand (sterilized previously), adding 20.0 +/- 0.1 ml of buffer according to determinable antibiotics, i.e. at determination of tetracycline - buffer N 3, zynbacitracin - N 4, grizin - saline solution (0.85% NaCl). In availability of

tissue micro breaker 20.0 +/- 0.1 ml of buffer were added to cutter finely divided sample, thoroughly mixed transferring into microbreaker glass and blended the sample at maximum velocity within 3 minutes. Antibiotics were extracted within 1.5 +/- 0.5 hours.

During study for grizin and zyncbacitracin, the homogenate was preheated in water bath at 65 +/- 5 degr. C within 30 minutes. For inactivation of possible inhibiting agents and better desorption of antibiotics.

Samples were centrifuged at 3000 rpm within 20 +/- 1 minutes

Supernatant fluid in the amount of 0.05 +/- 0.001 ml from eachtest specimen was added into 2-3 wells to 2 parallel Petri dishes. When determining grizin, dishes are placed into the fridge for 3 hours at +4 degr. C for predisersion.

Seeded dishes with supernatant fluid added into wells, are incubated within 18 +/- 0.5 hours at 29 +/- 1 degr. C (tetracyclines determination), or at 37 +/- 0.1 degr. C (determination of grizin and zyncbacitracin), thereafter diameters of zones of test-cultures growth retardation were measured and calculations of antibiotic residues activity in study substrates were made.

For microbiological analysis samples produced from different places from each carcass: the bottom of the shank, outer part of the thoracic

cut, the outer part of the butt. Samples were taken with a sterile knife from different parts of the carcass. Each sample of meat or 200 g wrapped in a clean parchment paper, which put the number. Several samples taken from a carcass, packaged in a paper bag before sealing, and sent to the laboratory. Carcass sampling for microbiological analysis was carried out by ISO 17604-2003. Salmonella spp determined according to GOST 30519-97 (GOST 50480-93), coliform bacteria (coliforms) was determined according to GOST 30518-97 (GOST 50474-93), the control method for the detection of Salmonella in ISO 3811-79, molds determined according to GOST 10444.12-88, bacteria QMAFAnM determined according to GOST 10444.15-94¹⁵.

RESULTS AND DISCUSSION

In accordance with findings of immunoenzyme method of analysis the following was determined in 3 muttons blood: cortisol concentration levels before killing were 153.3 nm/l, 229.0 nm/l and 239.5 nm/l, and during killing 355.6 nm/l, 411.3 nm/l and 569.4 nm/l, whereas housing and feeding conditions were similar. As can be seen in table 1 cortisol concentration contents in muttons blood before and during killing.

The higher stress is, the more cortisol,

Table 1. Cortisol concentration contents in muttons' blood (nm/l)

Mutton blood sample number	Actually received cortisol blood concentration, nm/l	Permissible standards, nm/l
R1 before killing	153.3	100-350
R2 before killing	229.0	100-350
R3 before killing	239.5	100-350
R1(1) during killing	569.4	100-350
R2(2) during killing	411.3	100-350
R3(3) during killing	355.6	100-350
R reference serum	372,6	100-350

Table 2. Antibiotics content in mutton meat

Antibiotics in meat, U/g	Permissible standards by ND	Actually received
Levomycetin	Non-perm. (<0.01)	Not detected
Tetracycline group	Non-perm. (<0.01)	Detected (<0.01)
Grizin	-	Not detected
Zyncbacitracin	Non-perm. (<0.02)	Not detected

Table 3. Results of microbiological analysis of raw meat

Indicator	Limit on Normative Documentation	Actual results	Method of analysis
1	2	3	4
1 sample. Mutton (n=5)			
Microbiological ind:	1*10 ³	<1,5*10 ²	GOST 10444.15-94
CMAFA m, NCU,			
Coliform bacteria 0,1 g	Not allowed	Not founded	GOST 30518-97
Patogenes, c.i. Salmonella, 25 g.	Not allowed	Not founded	GOST 30519-97
L monocytogenes, 25r.	Not allowed	Not founded	GOST R 51921-2002
2 sample. Beef (n=5)			
Microbiological ind:			GOST 10444.15-94
CMAFA m, NCU,	1*10 ³	1,5*10 ²	
Coliform bacteria 0,1 g	Not allowed	Not founded	GOST 30518-97
Patogenes, c.i. Salmonella, 25 g.	Not allowed	Not founded	GOST 30519-97
L monocytogenes, 25r.	Not allowed	Not founded	GOST R 51921-2002

the more cortisol – the longer strain of an organism, which means longer period of physical recovery.

On basis of data obtained during research, it can be supposed that stress predisposes cortisol hormone, in our situation, cortisol concentration in higher levels releases from the organism during killing, and this is exactly when meat becomes harmless and clean. In order to improve further animal productivity and receive high-quality meat is required favorable emotional environment¹⁶.

For the following stage of our work we foresee determination of cortisol in meat and animal fat using enzyme immunoassay methods and near IR spectrophotometer method and results can be seen in the Figure 1.

Sustained consumption of products containing antibiotic residues may cause adverse consequences for human health - hypersensitivity

reactions, disbacteriosis, development and transmission of resistant microbial forms as can be seen in table 2.

In accordance with laboratory findings for antibiotics contents in meat – mutton, we detected tetracycline group antibiotics (<0.01) within permissible standards, that is allowable in accordance with approved standards for production of halal products¹⁷.

The purpose of microbiological analysis is the detection of carcasses and counting pathogens causing infectious diseases among consumers. We have been carrying out microbiological analysis of the carcasses of sheep and cattle slaughtered by halal method. The results are shown in Table 3. The results of microbiological analysis shows compliance of samples analyzed by the number of combined units colimorfic-aerobic and facultative anaerobic microorganisms,

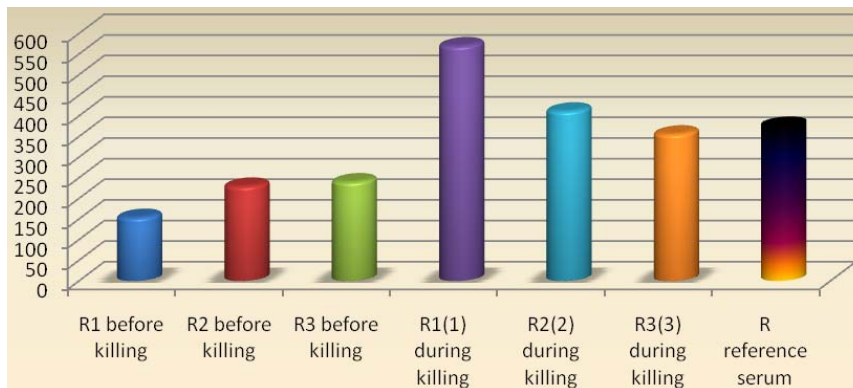


Fig. 1. Actually received cortisol blood concentration, nm/l

pathogens, including Salmonella admissible norm and their compliance with the limits of normative documents¹⁸.

In lamb and beef as raw meat materials in terms of CMAFA&M shall not exceed the allowable standard indicators, in this case does not exceed 1×10^3 , the actual value turned $<1.5 \times 10^2$. In all the samples of raw meat coliform bacterias and other pathogens weren't detected.

CONCLUSIONS

This study supports the notion that the higher stress is, the more cortisol, the more cortisol – the longer strain of an organism, which means longer period of physical recovery. On basis of data obtained during research, it can be supposed that stress predisposes cortisol hormone, in our situation, cortisol concentration in higher levels releases from the organism during killing, and this is exactly when meat becomes harmless and clean. In order to improve further animal productivity and receive high-quality meat is required favorable emotional environment during the peri-mortem stage. The results of microbiological analysis shows compliance of samples analyzed by the number of combined units colimorfic-aerobic and facultative anaerobic microorganisms, pathogens, including Salmonella admissible norm and their compliance with the limits of normative documents.

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