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Full Research Article

NUTRITIVE AND MICROBIAL QUALITY OF FEED FOR LAYING HENS FROM THE SERBIAN MARKET IN 2018

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Abstract

Introduction. Complete feed mixtures for laying hens are formulated to meet the requirements of the animals, for which various recommendations exist. Because of its nutritive value, feed provides a favorable environment for the growth of microorganisms. Prominent bacterial pathogens in poultry feed include *Salmonella, Escherichia coli, Enterococcus*, and *Clostridium*. There is a strong link between poultry feeds contaminated with bacteria pathogenic to humans and food borne disease occurrence, due to the feed-poultry-food-human chain.

Materials and Methods. A total of fifty samples of complete feed mixtures for laying hens were collected during 2018 as a part of official controls. Feeds were analyzed for crude protein, crude ash, crude fat, crude cellulose, calcium and phosphorus, total numbers of bacteria, numbers of molds, *Salmonella spp.* and sulfite-reducing clostridia.

Results and Conclusions. The feeds contained total bacteria and molds each at the level of 10^3 cfu g^{-1}. Additionally, no *Salmonella spp*. or sulfite-reducing clostridia were detected in any feed sample. Saprophytic bacteria are frequently found in complete feed mixtures, but rarely are present in numbers considered as microbiologically unsafe. However, the presence of saprophytic bacteria can decrease the nutritive value of feed. The total number of bacteria and molds below the regulatory limits, as well as absence of pathogens in the commercially available poultry feed, indicates a high level of compliance with regulation. Furthermore, these results indicate the adequate implementation of controls and supervision of these poultry feed products in the Serbian market.

Key words: laying hens, feed, nutritive quality, microbial safety

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INTRODUCTION

Complete feed mixtures are formulated to meet the requirements of poultry for growth and expressing maximum production performances (i.e. production of meat and eggs) (Sultana et al., 2017; Obi and Ozugbo, 2007). In poultry, the intestinal microbiota has a small role in feed digestion, which emphasizes the adequate nutritive value of feed that has to be easily digestible (Matthew et al., 2017; Smith et al., 2005). Complete feed mixtures for poultry are composed of plant feedstuffs (corn, wheat, barley, sunflower seeds) and animal origin feedstuffs (fish meal, meat or bone meal) (Sultana et al., 2017; Arotupin et al., 2007). Furthermore, complete feed mixtures contain inorganic sources of minerals and vitamins and various additives (probiotics, phytobiotics, organic acids, amino acid supplements, etc.).

There are different recommendations for laying hen feeds, set by NRC (1994), INRA-AFZ (2004), Serbian regulation (Official Gazette 4/2010, 113/2012, 27/2014, 25/2015, 39/2016) etc. Recommendations refer to the quantitative and qualitative values of feed, the chemical composition of feed (protein, dry matter, crude cellulose and carbohydrate content), but also refer to the vitamin and mineral contents and energy value of complete feed mixtures. Legislation also specifies the permissible numbers of microorganisms in feed and raw nutrients.

Feed is exposed to various biological, chemical and physical agents that pose a risk for animal and human health (Radanov-Pelagić et al., 2003; Hinton, 1993). Animal feed provides a favorable environment for the growth of microorganisms. Microorganisms in feed originate from normal feed microbiota, cross-contamination or water (Matthew et al., 2017). Furthermore, microorganisms can be deliberately added into the feed (in the case of probiotics).

There is a strong link between poultry feeds contaminated with bacteria pathogenic to humans and food borne disease occurrence, due to the feed-poultry-food-human chain. Prominent bacterial species that are of critical importance in poultry feeds include *Bacillus*, *Salmonella, Escherichia coli, Enterococcus, Campylobacter, Clostridium and Lactobacillus* (Sultana et al., 2017; Onyeze et al., 2013; Hossain et al., 2011; Nasrin et al., 2007).

The aim of this study was to give an insight into the nutritional value and quality, and to determine the microbiological safety of complete feed mixtures for laying hens present on the Serbian market. Results are also compared with regulatory standards.

MATERIALS AND METHODS

A total of fifty (50) samples of complete feed mixtures for laying hens in phase 2 of production (from 21 to 45 weeks) were collected during 2018 as a part of official

controls. Feed samples were analyzed for crude protein (ISO 5983/2001), crude ash (ISO 5984/2002), crude fat (ISO 6492/2001) and crude cellulose (ISO 6865/2004). Calcium (Ca) content in feeds was measured by flame atomic absorption spectrometry (ISO 6869/2008), and the phosphorus (P) content in feeds was determined by spectrophotometry (ISO 6491/2002).

Each feed (25 g amounts) was homogenized with buffered peptone water to obtain a 1:10 dilution. Homogenates were serially diluted to 10^{-5}. Total numbers of bacteria (ISO 4833-1:2014) and numbers of molds (ISO 21527-2:2011) were determined, and isolation and identification of *Salmonella* (ISO 6579-1:2017) and sulfite-reducing clostridia (ISO 7937:2010) were performed.

RESULTS

The average nutrient content of the analyzed feeds is presented in Table 1. Average values of nutrient contents in the feeds were in accordance with the local regulation. However, seven of the 50 feeds did not meet the nutritive value prescribed by local legislation for a single parameter. One feed had a percentage of crude protein below 15% (13.9%). Four feeds had a Ca content above 4.0%, two of which were above 4.5%, and three feeds had a P content below 0.6% (about 0.5%). Five feeds had an ash content above 13%.

Table 1. Nutrient content of feeds analyzed

Nutrient content of analyzed feeds, %		Number of invalid feeds that did not comply with Serbian regulations
Dry matter content	89.68±0.69	/
Crude ash	13.09±2.13	5
Crude protein	16.62±1.18	1
Crude fat	4.77±0.95	/
Crude cellulose	5.13±0.86	/
Calcium	4.01±0.61	4
Phosphorus	0.62±0.09	3

The average numbers of aerobic mesophilic bacteria and molds in the examined poultry feed mixtures are presented in Table 2. Total numbers of bacteria in the feeds varied from 1.00×10^{3} cfu g^{-1} to 3.80×10^{4} cfu g^{-1}, with an average number of 1.95×10^{4} cfu g^{-1} (Table 2). Mold numbers varied from 1.00×10^{4} cfu g^{-1} to 5.70×10^{4} cfu g^{-1} of feed, with an average number of 2.66×10^{4}cfu g^{-1} (Table 2).

Table 2. Microbiological quality determined for feeds from the Serbian market

	Total bacteria number in 1 g of feed	Total mold number in 1 g of feed	Presence of pathogenic microorganisms
Range of level of contamination	1.00×10^{3}-3.80×10^{4}cfu g^{-1}	1.00×10^{4}-5.70×10^{4}cfu g^{-1}	None detected
Average level of contamination	1.95×10^{4}cfu g^{-1}	2.66×10^{4}cfu g^{-1}	/

DISCUSSION

The quality of feed is assessed by its nutritive value, particle size, sensory and organoleptic properties, safety after consumption and microbiological quality (Gopi et al., 2017). The nutritive value and microbial quality are more important among these parameters. Serbian regulation determines the requirements for the nutritive quality of complete feed mixtures for laying hens (Official gazette, 4/2010, 113/2012, 27/2014, 25/2015, 39/2016).

In this study, 7/50 (14%) of analyzed feeds did not meet the nutritive requirement for a single parameter. One feed had an unsatisfactorily low content of crude protein. Crude protein is considered to be the most expensive nutrient, followed by energy value and phosphorus. Deficiency of protein in feed has adverse effects on poultry health, growth and production. On the other hand, environmental impacts (nitrogen excretion) can be a consequence of high dietary content of crude protein (Belloir et al., 2017). In the study conducted by Rahman et al. (2014), the crude protein content in feed varied more than in our study, and ranged between 11.91-27.29%. The fat content in feed reported by Rahman et al. (2014) ranged from 5.5 to 6.5%.

Calcium (Ca) and phosphorus (P) contents in complete feed mixtures for laying hens are important because of their impacts on the health and physiological status of the animals. These minerals are required for bone formation, as enzyme cofactors, for energy utilization and as structural components of cells (Humer et al., 2015; Proszkowiec-Weglarz and Angel, 2013). Ca and P are crucial in maintaining layer production performance at the highest level. Mineral status is crucial for egg production, egg weight, eggshell thickness and eggshell strength. In the current study, four feeds (8%) contained more Ca than the regulatory limit. Three feeds (6%) contained less P than the regulatory limit. Furthermore, the ratio of Ca to P in the feeds varied from 4.95:1 to 9.6:1. The literature recommendation for Ca:P ratio in feed for laying hens is 4:1, but in practical diet formulations, it varies in a wider range. However, an inadequate ratio of Ca to P affects the absorption and digestibility of these minerals. Furthermore, an excess of dietary Ca, and even more, an inadequate ratio of Ca to P, interferes with the availability of other minerals, such as magnesium, manganese, and zinc, as well as with the digestibility of dietary proteins (Woyengo and Nyachoti, 2013).

If it is considered that the recommendation for P intake refers to total P levels in feed, a lower content of this mineral in the feed must be considered important. However,

the level of digestible P contributes only part of the total P in feed. Even when the concentration of total P in feed is at the recommended level, the content of available P can be lower than that needed, as the animals' utilization of P will be insufficient (Pavlović et al., 2018).

Five feeds (10%) had ash contents above the regulatory limit, which was in line with the greater Ca contents in four feeds, because the ash, in the most part, comes from feed minerals, especially Ca. Rahman et al. (2014) determined ash content ranging from 6.34-18.85% in feeds.

One of the factors that determines feed safety is its microbiological quality. Poultry feeds can be contaminated in each phase of production (harvesting, processing, storage and retailing) directly or through contact with soil, rodents, birds, dust, human carriers, sewage or water (Sultana et al., 2017; Chowdhuri et al., 2011; Ezekiel et al., 2011). Microorganisms found in poultry feedstuffs can be saprophytic, pathogenic, conditionally pathogenic or toxic. Their and proliferation in the feed depends on numerous factors, including moisture, temperature, type of feed, aerobic and anaerobic conditions, chemical and physical properties of raw materials, feed pH, presence of feed additives, storage periods and conditions, and feed decomposition products (Đorđević et al., 2007; Radanov-Pelagić, 1999).

The feeds in the current study contained, on average, bacterial numbers of 10^3 cfu g^{-1}. Literature data about feed contamination are very variable. Sobczak et al. (2016) found the level of contamination of poultry feed was 10^4 cfu g^{-1}. Kwiatek et al. (2008) studied animal feeds, and found contamination by aerobic mesophilic bacteria at levels above 10^6 cfu g^{-1}, which occurred in 2.3-19.4% of the examined feed mixtures for poultry and 4.3-9.0% for livestock. A specific analysis of levels of contamination regarding the number of aerobic bacteria, conducted by Sobczak et al., (2016), stated that livestock feeds were less contaminated than poultry feeds. According to these authors, the recorded aerobic mesophilic bacteria levels in feeds were 10^3-10^6 cfu g^{-1}. Other research proved there were 10-fold higher average bacterial numbers in feed (Vlachou et al., 2004).

Saprophytic bacteria are frequently found in complete feed mixtures, but rarely are present in numbers that are considered as microbiologically unsafe (Official Gazette, 4/2010, 113/2012, 27/2014, 25/2015, 39/2016). However, the presence of saprophytic bacteria must not be neglected. In order to meet their own metabolic demands, these microorganisms utilize the substrate and so decrease the nutritive value of feeds. In adequate environmental conditions, they proliferate and alter the sensory properties of feed. Thus, they can indirectly affect the quality of feedstuffs and complete feed mixtures, regardless of maximum permissible limits.

The average number of molds in feeds in this study was at the level of 10^3 cfu g^{-1}. Sobczak et al. (2016) measured lower levels of feed contamination with mold, less than 10^2 cfu g^{-1}. On the other hand, Kwiatek et al. (2008) found contamination at much higher levels, above 10^5 cfu g^{-1} for 0.7-4.0% poultry feed. Čabarkapa et al. (2009)

found the number of molds in complete feed mixtures ranged from 0-10^5 cfu g^{-1}, with the exception of one feed in which the total number of molds was 5.00×10^5 cfu g^{-1}.

The total number of microorganisms in feed depends on many factors, among which the temperature and applied technological process during production are the most important. The efficiency of thermal conditioning in limiting and/or preventing microbiological contamination of feeds is proven in many bacteriological and mycological studies (Sobczak et al., 2016; De Vries et al., 2014). In complete feed mixtures, the technology of production implies thermal and other processing factors (drying, sterilization, extrusion, grinding, etc.) that contribute to the improved microbiological status. Thus, in complete feed mixtures, the level of microbial contamination can be much lower than in the raw ingredients, if hygienic measures and good agricultural and manufacturing practices are applied during all stages of production (growing, harvesting, drying and storage operations).

Pathogenic microorganisms – *Salmonella spp*. and sulfite-reducing clostridia – were not detected in the analyzed complete feed mixtures. Literature data are very variable regarding the presence of pathogens in poultry feed. Čabarkapa et al. (2009) detected sulfite-reducing clostridia in 18 feeds, but *Salmonella spp*. was not isolated from any feed. In another study, *Salmonella spp*. was not detected in feed (Okogun et al., 2016). On the other hand, Crump et al. (2002) stated that poultry feed is a major vector for transmission of *Salmonella* to poultry farms. *Salmonella spp*. was detected by Sultana et al. (2017) in poultry feed in Bangladesh, and by Wojdat et al. (2005) in Poland.

The presence of pathogens in feed can suggest subsequent contamination during processing or storage, as a consequence of some omission in the processing technology. Application of good agricultural and hygienic practices, along with measures to prevent microbial occurrence and growth in feed, will result in absence of pathogenic microorganisms. Upgrading of the feed industry regarding hygiene and feed treatment, as well as transport, packaging and storage of feed is crucial for prevention and control of microorganisms in feed (Sultana et al., 2017). Hence, tightly monitored and controlled production processes, during all phases, is crucial in order to obtain safe product.

CONCLUSION

The role of poultry feed in ensuring food safety and public health needs to be emphasized. The presence of bacteria and molds in feeds demands attention throughout the whole chain of production and sales, from feed mills to farms. It is imperative to carry out routine microbiological examination of poultry feed in order to monitor the microbial safety of feed. Implementation of good manufacturing and hygienic practice, along with monitoring the microbial safety of feed in each production phase (processing, storage, distribution and use) should improve quality and safety of animal feed. The absence of *Salmonella spp*. and sulfite-reducing clostridia

in commercially available poultry feed, as well as total numbers of bacteria and molds below the regulation limits, indicate compliance with local regulation. This high level of compliance with regulation point to the adequate implementation of control and supervision of poultry feed products placed on the Serbian market.

Authors contributions

MP worked on the results interpretation and text preparation. IP and MR worked on the layout of the manuscript according to the Journal's style and guidelines. SI did the proofreading of the manuscript, regarding to Journal's requirements for clear English language.

Competing interests

The authors declare that they have no competing interests.

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KVALITET I MIKROBIOLOŠKA ISPRAVNOST HRANE ZA NOSILJE SA TRŽIŠTA SRBIJE U 2018. GODINI

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Kratak sadržaj

Uvod. Potpune smeše za ishranu nosilja su formulisane da zadovolje potrebe za održavanje života i adekvatnih proizvodnih performansi, a za koje postoje različite preporuke. Zahvaljujući visokoj nutritivnoj vrednosti hrana za životinje predstavlja povoljan supstrat za rast mikroorganizama. Bakterijske vrste koje se najčešće dovode u vezu sa hranom za živinu su *Salmonella, Escherichia coli, Enterococcus, and Clostridium*. Hrana kontaminirana patogenim bakterijama, može dovesti do pojave oboljenja i kod ljudi, kroz lanac hrane.

Materijal i metode. 50 uzoraka potpunih smeša za ishranu nosilja je uzorkovano tokom 2018. godine kao deo službene kontrole. Uzorci hrane su analizirani na sadržaj proteina, pepela, masti, celuloze, kalcijuma i fosfora, broj aerobnih kolonija, ukupan broj gljivica i plesni, *Salmonella spp.* i sulfitoredukujuće klostridije.

Rezultati i zaključak. Ukupan broj bakterija i plesni u uzorcima hrane za živinu bio je na nivou od 10^3 cfu/g. *Salmonella spp.* i sulfitoredukujuće klostridije nisu izolovane iz ispitivanih uzoraka. Saprofitske bakterije su često prisutne u potpunim smešama za ishranu životinja, ali retko u broju zbog kojeg bi se ova hrana smatrala mikrobiološki neispravnom. Saprofitske bakterija mogu smanjiti nutritivnu vrednost hrane za životinje, koristeći je kao susptrat za sopstvene metaboličke potrebe. Određeni ukupan broj bakterija i plesni, kao i odsustvo patogenih mikroorganizama u ispitivanim uzorcima

ukazuje na usklađenost proizvođača sa zakonskom regulativom, uz adekvatnu kontrolu i nadzor nad proizvodnjom i prometom hrane za živinu u Republici Srbiji.

Ključne reči: koke nosilje, hrana, kvalitet, mikrobiološka ispravnost