Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 53, 2014: 13–20
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 53, 2014)

Effect of rearing system on the microbiological quality of Pekin P44 and Muscovy MR71 ducks bowel

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Abstract: *Effect of rearing system on the microbiological quality of Pekin P44 and Muscovy MR71 ducks bowel*. In this study, we analyzed the effect of rearing system of ducks on the quantitative and qualitative composition of their gut microflora. 180 ducks and 180 drakes of Pekin 44 and Muscovy MR71 lines were kept in the intensive system on litter; the same number of birds was kept in the semi-intensive system. In the latter, starting from the 3rd week of life the birds were allowed to use free ranges. After slaughter of 15 ducks and 15 drakes from each group, their intestinal digesta was subjected to microbiological analysis. The possibility of using the free ranges had a positive effect on the quantitative and qualitative composition of intestinal microflora of these birds. The count of aerobic mesophilic heterotrophs in the intestinal digesta of ducks was higher in the case of birds kept in the semi-intensive system. More beneficial results were achieved in the case of Muscovy ducks considering mainly the coli/lacto ratio which was the lowest (0.08) in this group for both sexes kept on free range. Noteworthy is also that no pathogenic bacteria of the genera: *Salmonella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Clostridium perfringens* and *Escherichia coli* were found in any of the analyzed samples.

Key words: Pekin ducks, Muscovy ducks, bowel, microbiological quality, rearing system

INTRODUCTION

Owing to the ban on the use of antibiotic growth stimulants in poultry feeding imposed on the 1 January 2006 in the EU Member States, the maintenance of the appropriate microbiological medium of the gastrointestinal tract is a prerequisite of the production cycle. Intestinal bacteria have a great impact on effective utilization of nutrients, functions of the immune system and, thereby, on the development of the whole body of birds (Kohl 2012). Irrespective of species, it is assumed that chicks hutch with sterile gastrointestinal tract that is next colonized by microorganisms inhabiting their environment. According to Cook et al. (2005), Peralta-Sa´nchez et al. (2010) and Ruiz-De-Castan˜eda et al. (2011), under natural conditions a great role is ascribed in this process to hutching behavior of parents and their microbiological status. In the production process, however, the role of adult birds was limited only to the production of hatching eggs. As a consequence, the microbiological status of chicks is influenced mainly by conditions occurring in the hatching incubator and in the rearing facility, meaning indirectly by man's actions.

Development of methods for the intensive production of poultry, with

the main assumption of as maximal as possible isolation of the area the birds are kept at (constant keeping the birds indoors) from impacts of the external environment as well as dissemination of the so-called principles of bio-protection, have in part created the possibility of controlling the development of the appropriate microflora in the gastrointestinal tract of poultry. Today, production of this type is most frequently condemned by animal rights defenders and consumers as it diminishes the welfare of birds (Fanatico et al. 2008) and deteriorates the quality of finished products (Berri et al. 2005). The rearing of birds in semi-intensive, ecological systems which allow the birds to use free ranges for at least 1/3 of their life span (Council Directive No 2092/91/EEC on 24 June 1991/Rozporządzenie Rady nr 2092/91/EWG z dnia 24 czerwca 1991 roku) is becoming an alternative to this system. A question then arises whether birds produced via artificial incubation and kept indoors at least in the initial period of life are capable to cope with and yield effective production results when allowed to use free ranges. It needs to be emphasized that even upon the greatest caution of man, it is impossible to isolate slaughter poultry from wild fowl and to control microclimatic conditions, as well as to avoid changes in the total administered feed as affected by ingestion of fresh vegetation by birds at the pasture.

This study was aimed at analyzing the effect of rearing system (intensive – IS, semi-intensive – OS) on the microbiological quality of bowels of two types of functional ducks in Poland: Pekin P44 and Muscovy MR71.

MATERIAL AND METHODS

Experimental procedures were approved by the Ethical Commission (approval no. 27/2009 of 16 April 2009).

The study was conducted with Muscovy ducks (Grimaud, Rossy, France) of R71 line (MR71) (Grimaud Fréres 2012) and Polish Pekin ducks of P44 line (P44) (Kokoszyński et al. 2010). Till the 3rd week of life, 360 ducks from each group (MR71: 180 ♂♂ and 180 ♀♀; P44: 180 ♂♂ and 180 ♀♀) were kept in the intensive system on litter in the same building (2.9 bird/m^2), afterwards half of ducks and drakes randomly selected from each group were allowed to use free ranges (0.08 bird/m^2) – Figure 1. Both sexes of P44 ducks were reared till the 7th week of life, whereas MR71 ducks till the 10th and MR71 drakes till the 12th week of life, however chick inclusions were made at various terms in order to synchronize slaughter time. Detailed data related to the characteristics of free ranges, weather conditions occurring when the birds were using free ranges, nutritive values of feed mixtures and basic production results including: growth, feed conversion ratio and mortality, were published in our earlier work (Damaziak et al. 2014).

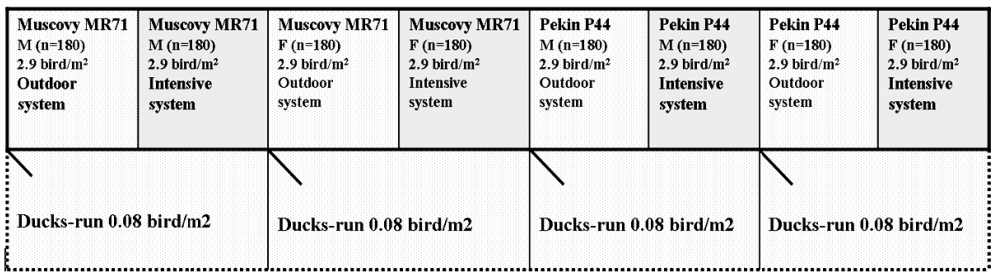

FIGURE 1. Experimental scheme showing the location of pens (—) and runs (·····) for experimental birds

After slaughter, samples of small intestine digesta were collected from 15 ducks and 15 drakes from each groups (in total: 120 birds) for microbiological analyses. Determinations were carried out for: ammonifying heterotrophic bacteria (nutrient agent without/with the addition of 5% defibrinated ram blood at 37°C); bacteria (lac+ and lac–) of the family Enterobacteriaceae (culture medium according to McConkey'(at 37°C); bacteria of the genus *Pseudomonas* – culture medium according to King B (28°C); microscopic filamentous fungi and yeast – culture medium according to Martin and Sabouraud with the addition of streptomycin (28°C); lactic fermentation bacilli of the family *Lactobacillus* – solid culture medium according to Eijkman with glucose, lactose and bromocresole purple and Sabouraud's medium (without antibiotics) (37°C); and sulfate-reducing *Clostridium perfingens* bacteria – liquid medium and solid medium according to Wilson-Blair (37°C). The count of bacteria was determined with the plate method and test tube method (MPN – most probable number).

Microorganisms were identified based on macroscopic observations of liquid and solid cultures and microscopic preparations of life bacteria in a flat droplet, stained with the Gram's method and with acridine orange.

The statistical analysis of results was conducted using statistical package SPSS 21.0 (IBM SPSS 2012). Normality of variables distribution was checked with Kołmogorow-Smirnow test. The contribution of ammonifying heterotrophs and Enterobacteriaceae family bacteria (lac+) showed normal distribution after logarithmic transformation (decimal logarithm from the variable + 1). The Lac – variable did not show normal distribution, even after transformation, hence non-parametric Mann-Whitney test was used in its case. The effect of factors on other parameters, which showed normal distribution, was estimated with analysis of variance (GLM procedure). The model of variance analyses for variables possessing normal distribution was as follows:

$$Y_{ijmk} = \mu + P_i + U_j + R_m + (PU)ij + (PR)im + (UR)jm + (PUR)ijm + e_{ijmk}$$

where:

Y_{ijmk} – trait;
μ – general mean;
P_i – effect of i-th sex, $i = 1.2$;
U_j – effect of j-th housing system;
R_m – effect of m-th genotype, $m = 1.2$;
$(PU)ij$ – effect of interaction between sex and housing system;
$(PR)im$ – effect of interaction between sex and genotype;
$(UR)jm$ – effect of interaction between housing system and genotype;
$(PUR)ijm$ – effect of interaction between sex, housing system and geno type;
e_{ijmk} – random error.

RESULTS AND DISCUSSION

Based on study results presented in Table 1, it was stated that the count of aerobic mesophilic heterotrophic bacteria in small intestinal digesta of ducks was significantly ($P \leq 0.01$) higher in the case of birds kept in the OS system, compared to the IS system. This was, probably, linked with the possibility of ingesting green vegetation by birds. Gajewska et al. (2009) demonstrated earlier the feeding chickens mixtures with the addition of plant preparations containing mixtures of various herbs significantly increased the count of aerobic microorganisms. In addition, a reduction was observed in the count of bacteria of the family Enterobacteriaceae, grown on McConkey's medium, in both types of ducks produced in the OS system (Table 1). The use of Sabouraud's medium and Eijkman's medium for the culture of lactic fermentation bacteria did not cause any significant differences in bacterial counts between ducks kept in different rearing systems. A higher count of *Lactobacillus* bacteria was determined only in the case of MR71 ducks reared in the OS system, but the significance was confirmed at $P \leq 0.05$ (Table 1). It is known that the basic microorganisms for birds are facultative and strict anaerobic bifidobacteria, *Lactobacillus* and lactate--fermentation bacteria, and *Bacteroides* (Yaghobfar et al. 2006).

No pathogenic bacteria of the genera: *Salmonella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Clostridium perfringens* and *Escherichia coli* were found in any of the analyzed samples. No filamentous fungi and yeast were either detected with the use of Martin's medium, irrespective of the rearing system, origin and sex of birds (Table 1). According to Ziółkowska and Tokarzewski (2007), an increase of infection rate with *Salmonella* spp., *Pasteurella* spp., *Campylobacter* spp. and *Listeria* spp., was observed in geese as a result of progressing intensification of the production process and increasing stock density of birds. The lack of these microorganisms in digesta of ducks in this study could be due to rearing conditions (OS groups), as well as

TABLE 1. Total count of aerobic heterotrophs, filamentous fungi and yeast (LSM; SEM) in digesta of small intestines of Pekin P44 and Muscovy MR71 ducks (1 g d.m.), reared on litter (IS) and on free ranges (OS)

Housing system	Genotype	Sex	Sabouraud *Lactobacillus*	Eijkman *Lactobacillus*	Nutrient agar[a] Endo_lac+/ /lac–	McConkey Enterobacteriaceae	Martin[b] fungi and yeast
OS	P44	M	2.48×10^5	1.11×10^5	3.09×10^7	3.21×10^4	$<1.0 \times 10^2$
		F	3.17×10^5	2.00×10^5	3.27×10^7	3.00×10^4	$<1.0 \times 10^2$
	MR71	M	2.33×10^5	1.02×10^5	0.12×10^7	5.51×10^2	$<1.0 \times 10^2$
		F	3.01×10^5	1.00×10^5	2.21×10^7	4.89×10^3	$<1.0 \times 10^2$
IS	P44	M	2.20×10^5	1.87×10^4	2.25×10^6	2.52×10^5	$<1.0 \times 10^2$
		F	0.66×10^5	0.36×10^4	6.67×10^5	2.40×10^5	$<1.0 \times 10^2$
	MR71	M	2.05×10^3	4.3×10^5	3.34×10^6	1.93×10^5	$<1.0 \times 10^2$
		F	1.19×10^4	3.9×10^5	3.23×10^6	2.20×10^5	$<1.0 \times 10^2$
SEM			0.20	0.05	0.49	0.75	0.00
Main effects							
housing system			*	NS	**	**	NS
genotype			*	*	**	**	NS
sex			*	NS	NS	*	NS
housing system × × genotype			NS	*	**	**	NS
housing system × × sex			*	NS	**	**	NS
genotype × sex			NS	NS	**	**	NS
housing system × × genotype × sex			NS	NS	**	**	NS

[a] Nutrient agar with the addition of ram blood; [b] filamentous fungi and yeast; NS: $P > 0.05$, *$P \leq 0.05$, **$P \leq 0.01$; SEM – standard error of the mean.

to low stock density and relatively low number of birds in experimental flocks.

The housing system was observed to have a significant effect on changes in the coli/lacto ratio computed based on the ratio of total count of Enterobacteriaceae family bacteria to the count of LAF bacteria (Table 2). In the case of both sexes of MR71 ducks, a more favorable, lower (ca. 8 times lower) value of the ratio was noted for ducks from the OS system, compared to birds produced in the IS system. In the case of P44 ducks, values of the coli/lacto ratio did not differ significantly between birds reared in the IS and OS systems. Similar values were noted for birds of both sexes. Probably, the lowest values of the coli/lacto ratio determined for MR71 ducks produced in the OS system are linked with longer rearing period of these birds and, thereby, with longer period when the birds could use free ranges, compared to P44 birds.

TABLE 2. Values of coli/lacto ratio determined based on counts of Enterobacteriaceae family bacteria and LAF bacteria (LSM; SEM), present in digesta of small intestines of Pekin 44 and MR71 Muscovy ducks, reared on litter (IS) and on free range (OS)

Housing system	Genotype	Sex	Endo_lac+	Endo_lac–	Lactobacillaceae	Coli/lacto
OS	P44	M	5.51×10^6	0.01×10^5	2.30×10^6	0.52
		F	1.40×10^7	0.11×10^6	3.12×10^7	0.65
	MR71	M	2.15×10^6	0.00×10^5	2.30×10^6	0.08
		F	2.20×10^6	0.00×10^5	2.79×10^6	0.08
IS	P44	M	9.66×10^6	0.01×10^5	2.12×10^7	0.66
		F	3.36×10^6	0.01×10^5	0.57×10^6	0.74
	MR71	M	2.70×10^6	0.31×10^5	1.84×10^7	0.59
		F	6.64×10^6	0.24×10^6	1.16×10^7	0.54
SEM			0.17	0.00	0.58	0.05
Main effects						
housing system			**	**	*	**
genotype			**	NS	NS	*
sex			NS	NS	NS	NS
housing system × genotype			**	**	NS	**
housing system × sex			**	**	*	**
genotype × sex			**	*	NS	**
housing system × genotype × × sex			**	NS	NS	NS

NS: $P > 0.05$, *$P \leq 0.05$, **$P \leq 0.01$; SEM – standard error of the mean.

The housing system was observed to have a significant effect on changes in the coli/lacto ratio computed based on the ratio of total count of Enterobacteriaceae family bacteria to the count of LAF bacteria (Table 2). In the case of both sexes of MR71 ducks, a more favorable, lower (ca. 8 times lower) value of the ratio was noted for ducks from the OS system, compared to birds produced in the IS system. In the case of P44 ducks, values of the coli/lacto ratio did not differ significantly between birds reared in the IS and OS systems. Similar values were noted for birds of both sexes. Probably, the lowest values of the coli/lacto ratio determined for MR71 ducks produced in the OS system are linked with longer rearing period of these birds and, thereby, with longer period when the birds could use free ranges, compared to P44 birds.

In summary, the results obtained indicate that allowing ducks to use free ranges had a positive effect on the qualitative and quantitative composition of their gut microflora. Of the two genetic groups of ducks (P44 and Muscovy), better microbiological parameters were noted in the case of MR71 OS group,

which could be due to the longer period of rearing and using free ranges. In the future, results of microbiological assays of intestinal digesta should be confronted with results of parasitological analyses, since the risk posed by internal parasites is one of the major causes postulating for reduced application of the semi-intensive methods of poultry rearing.

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Streszczenie: *Wpływ systemu utrzymania na jakość mikrobiologiczną jelit kaczek Pekin P44 i piżmowych MR71*. W badaniach analizowano wpływ systemu utrzymania kaczek na ilościowy i jakościowy skład mikroflory jelitowej. Po 180 kaczek i 180 kaczorów Pekin P44 i piżmowych MR71 utrzymywano w systemie intensywnym na ściółce i taką samą ilość ptaków w systemie półintensywnym. W systemie półintensywnym ptaki od 3. tygodnia życia mogły korzystać z ograniczonych wybiegów. Po uboju 15 kaczek i 15 kaczorów z każdej grupy wykonano analizę mikrobiologiczną treści ich jelit cienkich. Możliwość korzystania z wybiegów miało pozytywny wpływ na jakościowy i ilościowy skład ich mikroflory jelitowej. Liczebność tlenowych mezofilnych bakterii heterotroficznych, w treści jelita

cienkiego kaczek, była wyższa dla ptaków utrzymywanych w systemie półintensywnym. Korzystniejsze wyniki stwierdzono w przypadku kaczek piżmowych, głównie odnośnie współczynnika coli/lacto, który był najniższy (0,08) u tej grupy dla obu płci utrzymywanych na wybiegu. Na szczególną uwagę zasługuje również fakt, iż nie stwierdzono obecności bakterii chorobotwórczych z rodzaju *Salmonella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Clostridium perfringens* i *Escherichia coli* w żadnej z badanych prób.

MS. received in November 2014

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