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Original article

Influence of the age of turkey layers on selected serological parameters and the transfer of maternal antibodies to chicks hatched from their eggs

M. Wegner[2], D. Kokoszyński[1], J. Frischke-Krajewska[3], J. Bujko[4], A. Żurek[2]

[1] Department of Animal Breeding and Nutrition, Faculty of Animal Breeding and Biology, Bydgoszcz University of Science and Technology, Mazowiecka 28, 85-084 Bydgoszcz, Poland
[2] Boehringer-Ingelheim, J.P. Dziekońskiego 3, 00-728 Warsaw, Poland
[3] Vetdiagnostica Sp. z o.o., Otorowo 30, 86-050 Solec Kujawski, Poland
[4] Institute of Nutrition and Genomics. Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, 94-976 Nitra, Slovakia

Correspondence to: D. Kokoszyński, e-mail: kokoszynski@gmail.com

Abstract

The purpose of this study was to compare the effect of the age of the breeder flock of commercial BUT – 6 turkeys on the transfer of maternal antibodies to chicks. The blood samples for serological analysis were collected from randomly selected 63 female breeders from a flock of BUT Big 6 turkeys and 63 one-day-old hybrid turkey poults hatched from eggs from this flock at 36, 45 and 54 week of age. During blood analysis (serum) in the laboratory, the level of antibodies of the breeder flock against Avian metapneumoviruses (APV), Newcastle disease virus (NDV) and Hemorrhagic enteritis virus (HEV) was determined (ELISA). Maternal antibody (MatAb) titer in chicks (serum) against the same viruses were also determined. The percentage (%) transfer of MatAb to offspring was then evaluated. The effect of the age of the turkeys on the antibody titer to the tested pathogens expressed in geometric mean titers (GMT) was shown. During the laying period, the antibody titer of the tested turkeys against NDV decreased with the age of the flock. The highest antibody titer was demonstrated in week 36 (GMT=14242), whereas the lowest was in week 54 (GMT=5564). In contrast, the serum antibody titer of the tested layers against APV and HEV increased with the age of the birds. The lowest antibody titer (GMT_{APV}=24818; GMT_{HEV}=12070) was observed at the beginning of the laying period, and the highest at the end of the laying period (GMT_{APV} =38978; GMT_{HEV} =13980). The highest vertical transfer to offspring was shown for antibodies to – HEV (82.7%), while the lowest was shown when analyzing sera to – NDV (37.6%). The present analysis showed significant differences in the evaluated antibody titres in serum of turkey breeders during the laying period, as well as in the level of MatAb in chicks. The results also indicate that the transfer of MatAb to chicks is influenced by the age of the parent flock and the type of pathogen against which the layers were vaccinated.

Keywords: humoral immunity, infection diseases, maternal antibody transfer, turkey layers, turkey poult

Introduction

Preventive vaccinations of poultry are very important as they protect birds against infection by virulent pathogens, which are present in their environment and have a negative effect on mortality levels, weight gains, feed intake, and the number of eggs produced (Lemiere 2013, Panth 2019, Wegner et al. 2024). A well-protected parent flock helps ensure proper development and no health issues but, most of all, it has an effect on the health of poults through a vertical transfer of MatAb (Murai 2013). This is why preventive vaccinations of parent flocks are carried out, which are supposed to trigger an immune response by exposing the immune system to contact with vaccine antigens. Turkey layers are most often protected against NDV, HEV and APV. One of the most dangerous diseases of the upper respiratory tract is infection with avian metapneumovirus (APV), which has been described as turkey rhinotracheitis (TRT), affecting mainly turkeys. The disease, diagnosed in chickens in 1984, was named swollen head syndrome (SHS) by Thomson and Morley (Kaobudi and Lachheb 2021).

The immune system in birds produces 3 classes of antibodies: IgY, IgA, and IgM (Leslie and Clem 1969, Chung et al. 2020). Leslie and Clem (1969) demonstrated that IgY immunoglobulins may be found predominantly in the yolk; whereas IgA and IgM antibodies are present in albumen. The amounts of antibodies in the yolk and albumen are different. There are significantly fewer IgA and IgM antibodies in the albumen than there are IgY antibodies in the yolk (Rose et al. 1974, Dohms et al. 1978).

Antibodies are transferred from the layer onto her offspring in two stages. At the first stage, IgY antibodies are captured by IgY receptors on the ovarian follicle from the layers's blood, and they are transferred to the yolk. The next step is when immunoglobulins are supplied from the yolk to the offspring through embryonic blood circulation (Cutting and Roth 1973, Loeken and Toth 1983). Other research has shown that the speed of IgY transfer from the yolk sac to the embryonic blood circulation starts to increase between days 14 and 21 of the development of the chicken embryo (Kowalczyk et al. 1985). However, IgA and IgM antibodies pass from the fallopian tube to the albumen, and then, during the development of the embryo, a small amount reaches the yolk sac and amniotic fluid. In the next stage, they are transported to the intestines together with the amniotic fluid, as opposed to IgY immunoglobulins – which reach the embryo's blood (Higgins 1975, Rose and Orlans 1981, Kasper et al. 1991, Kasper et al. 1996). Other research has demonstrated that the IgY immunoglobulin level is a direct indicator of MatAb transfer to poults (Hammal et al. 2006).

However, one-day old birds, do not have a fully developed immune system, hence it is particularly important to protect poults from various pathogens via MatAb. Mondal and Nagi (2001) demonstrated a very high level of MatAb in poults at 1 day of age, which provided a high protective barrier against the infectious bronchitis virus (IBV); while the antibody level on day 7 was at only 30%. In other research, it was shown that the level of MatAb against the infectious bursal disease virus (IBDV) in poults remained stable until day 21, whereas antibody levels for other antigens were retained only until day 10 (Ahmeda and Akhtera 2003, Gharaibeh and Mahmoud 2013).

Many studies have been conducted on the MatAb level in poults with regard to various pathogens: NDV (Rahman et al. 2002, Kjaerup et al. 2017, Dobner et al. 2019, Kowalczyk et al. 2019, Chung et al. 2020), *Campylobacter jejuni* (Sahin et al. 2001), *Escherichia coli* (Heller et al. 1990), IBV (Mondal and Nagi 2001, Nari et al. 2020) and Turkey arthritis reovirus (TARV) (Kumar et al. 2022). It has been demonstrated that in the case of some viruses causing avian encephalomyelitis (AEV), as well as chicken anaemia virus (CAV) or reovirus (Reo), the MatAb level in poults is key, since it provides the only protective barrier (van der Heide et al. 1976, Westbury and Sinkovic 1978, Otaki et al. 1992). In another study, it was shown that MatAb in poults, which are transferred vertically onto the offspring, can reduce replication of the *Mycoplasma gallisepticum* (MG), thus decreasing poult mortality (Levisohn et al. 1985). This is why preventive vaccinations of the breeder flock are so important; they can protect poults in the early days of their lives.

The aim of our research was to determine the correlation between the level of antibodies in the parent flock to the transfer of MatAb to the offspring during the laying period against selected pathogens (APV, NDV, and Hemorrhagic enteritis virus – HEV).

Materials and Methods

The source of the results obtained was laboratory to which the veterinarian sent the blood samples. The blood samples were collected from the turkeys during flock health monitoring throughout the laying period we analysed. There were no additional interventions on the animals and therefore ethics committee approval was not required, as stipulated in the Polish Animal Protection Policy (35), which is in line with European Union Directive 2010/63/EU on the protection of research animals.

Table 1. Vaccination schedule for the BUT 6 turkey breeder flock.

Age	Disease	Serotype/strain	Typ of vaccine	Method of vaccination
1 d	TRT	serotype B	live	coarse spray
3 wk	ND	Ulster 2C	live	coarse spray
4 wk	HE	Domermuth	live	drinking water
5 wk	TRT	serotype A	live	aerosol
7 wk	ND	Ulster 2C	live	drinking water
12 wk	TRT	serotype B	live	aerosol
16 wk	ND+TRT+PMV3	Ulster 2C/ VCO3/ AAvV-3	inactivated	intramuscular injection
21 wk	HE	Domermuth	live	drinking water
27 wk	ND+TRT+PMV3	Ulster 2C/ VCO3/ AAvV-3	inactivated	intramuscular injection
27 wk	TRT	serotype A	live	aerosol

TRT – *Turkey rhinotracheitis*, ND – *Newcastle disease,* PMV3 – *Paramyxovirus type 3*, HE – *Hemorrhagic enteritis*

Animals and farm environment

The blood samples for serological analysis were collected from 63 randomly selected female breeders from a flock of BUT Big 6 turkeys and 63 one-day-old hybrid turkey poults hatched from eggs from this flock. The parent turkeys were selected from a flock of 4,000 females from a commercial farm in Poland. Rearing was continued until week 28 of life, during which the environmental parameters (temperature, air humidity, ventilation), and feeding were regulated as recommended (Management Essential for Breeder Turkeys 2016) and based on daily observations, which were used to make adjustments to existing conditions. In the course of rearing, a basic vaccination schedule was applied (Table 1).

The birds were then transported to a commercial farm. During the laying period, layers and turkey males were kept in a turkey house, where they received separate fodder as recommended (2016) and had unlimited access to water; exposure to light was 14.5 hours/day. The birds were kept on straw, and the turkey houses were equipped with manual nests – 4 layers per nest. The temperature inside the turkey houses was kept at 16-17°C, relative humidity in the laying period was 50% to 70%, and light intensity was 40 lux.

Serological analysis

At 36, 45, 54, weeks of age, during routine veterinary inspection (flock-health monitoring), blood samples were collected from 63 turkeys (21 on each evaluation date) for analysis. The blood samples were delivered to the commercial accredited veterinary diagnostic laboratory (SLW BIOLAB, Ostróda, Poland). The titers against NDV, APV, HEV were then determined in the blood serum, and the geometric mean titer levels (GMT) of the above pathogens were determined. After the chicks hatched, (21 birds from each test date) blood samples were taken from 63 randomly selected individuals. The samples were delivered to the SLW BIOLAB laboratory for determination (in serum) of MatAb titers. The tests were performed using a commercial ELISA kit (APV – BioCheck UK, NDV – IDEXX NDV-T-Ab, Netherlands, HEV – ProFLOK, HEV-T Ab, Zoetis, USA), which was used to determine the titer (MatAb) against APV, NDV, and HEV. ELISA kits were used according to the manufacturer's protocol. The antibody titer for each bird was quantified using software provided by the manufacturers (xCheck, BioCheck, and ProFile3) software. GMT for each group of serum samples were also calculated using the same software. All serum samples were read against the provided antisera, positive and negative controls.

Percentage transfer of antibodies against NDV, APV, and HEV was calculated based on the formula (MatAb level of chicks/ antibody titer of turkeys*100%), during the aforementioned study periods.

Statistical analyses

Collected numerical data were described with the use of generally approved methods of mathematical statistics. Applying the SAS package [SAS Institute, Cary, NC, USA], we calculated mean values (x) and standard deviations (SD) of the examined traits, was than performed analysis of variance , and the significance of differences was assessed using the Tukey's test. Significance of differences was verified at the $p \leq 0.05$ level.

Results

Serological analysis

Analyzing the results of serological investigations of turkeys (Table 2) showed significant ($p \leq 0.05$) differences in the antibody titer of the breeder flock during

Table 2. Serum antibody titers in BUT 6 reproductive turkeys at various laying periods.

Parameter	36 wks*	45 wks	54 wks	P-*Value*
APV	24818[b] ± 12703	37124[a] ± 10218	38978[a] ± 12489	0.0004
NDV	14242[a] ± 5847	8330[b] ± 6250	5564[b] ± 2994	<0.0001
HEV	12070[b] ± 1625	12697[b] ± 1908	13980[a] ± 1079	0.0008

Values are expressed as the geometric mean (± SD)
APV – *Avian pneumovirus;* NDV —*Newcastle disease virus;* HEV – *Hemorrhagic enteritis virus*
[a,b] mean values marked with different letters in columns differ significantly (p≤0.05)
* age of the parent flock

Table 3. Serum antibody titers in 1-day-old turkey poults depending on the age of turkey layers.

Parameter	36 wks*	45 wks	54 wks	P-*Value*
APV	10155[b] ± 7529	20302[a] ± 9568	23152[a] ± 12673	0.0002
NDV	5243[a] ± 2738	3181[b] ± 2237	2108[b] ± 1497	<0.0001
HEV	11082 ± 1919	10864 ± 2048	9895 ± 2080	0.1362

Values are expressed as the geometric mean (± SD)
APV – *Avian pneumovirus*, NDV – *Newcastle disease virus*, HEV – *Hemorrhagic enteritis virus*
[a,b] mean values marked with different letters in columns differ significantly (p≤0.05).
* age of the parent flock.

Table 4. Percentage (%) of antibodies transfered from turkey layers to poults.

Parameter	36 wk*	45 wk	54 wk	mean
APV	40.9	54.7	59.4	51.7
NDV	36.8	38.2	37.9	37.6
HEV	91.8	85.6	70.8	82.7

APV – *Avian pneumovirus*, NDV – *Newcastle disease virus*, HEV – *Hemorrhagic enteritis virus*
* age of the parent flock

the entire laying period against the tested viruses expressed in GMT. The highest level of anti-HEV (GMT=13980) was found in females at 54 weeks of age, while the lowest level (GMT=12070) was found at 36 weeks. The same correlation was found by analyzing the mean titers against APV. The lowest (GMT= 24818) titers were found in layers at 36 weeks, and the highest (GMT=38978) at the end of the laying period. Analysis of the titer of antibodies against NDV in the serum in turkeys showed a decrease in the antibodies during the laying period (P<0.0001). The highest level of antibodies expressed in GMT was shown at 36 weeks (GMT=14242), while the lowest was at 54 weeks (GMT=5564).

Analysis of the results of serological investigations of chicks showed statistically significant differences (p≤0.05) in the level of anti-APV and anti-NDV antibodies (Table 3). During the analyzed periods, significantly higher anti-APV antibody titres were found in the serum of chicks hatched from eggs obtained at 45 and 54 weeks (GMT=20302, GMT=23152, respectively) than in chicks derived from eggs collected at the beginning of the laying period (GMT=10155). In the case of serum testing for anti - NDV, it was shown that the highest average antibody level (GMT=5243) was in chicks hatched from eggs collected at 36 weeks of age of females, and statistically (p<0.0001) the lowest (GMT=2108) at the end (54 weeks) of the laying period of the breeder flock. Antibody titer in chicks decreased with the increasing age of the breeder flock, in which antibody titer also decreased with age. Analysis of the quantitative level of MatAb in chicks tested for – HEV showed no significant differences (p=0.1362), but the number of antibodies in the serum of chicks tested decreased slightly with the increasing age of females.

During the entire laying period, the average vertical transfer of antibodies from layer to chick (Table 4) ranged from 37.6% to 82.7%. The highest transfer was determined when anti-HEV sera were tested. It was shown that with the age of females, the percentage of transferred antibodies (anti-HEV) to offspring decreased from 91.8% – 36 weeks to 70.8% – 54 weeks. Analyzing the percentage transfer of anti-APV antibodies from parents to offspring showed increase during the laying period. At the peak of laying (36 weeks), the percentage of antibodies transferred was 40.9%. The average percentage of transferred anti-APV antibodies for the entire period analyzed was 51.7%. During the entire laying period, the transfer of specific anti-

bodies (NDV) was at a similar level (37.6%), and the age of females did not affect the percentage decrease in antibody transfer to offspring.

Discussion

Preventive vaccinations in poultry are intended to induce an immune response (immunity) to a specific type of pathogen or disease-causing agent (Suardana et al. 2023). Antibodies obtained as a result of vaccination of laying layers are transferred vertically in different percentages to the offspring depending on the disease (Ali and Hasan 2018). The level of antibodies in parental flocks and chicks is influenced by the age of the birds, breeding line, genotype and MAtAb transfer (Abdel-Moneima and Abdel-Gawada 2006, Kjaerup et al. 2017, Ali and Hasan 2018, Dobner et al. 2019, Kowalczyk et al. 2019, Abdelhamid et al. 2024). In our study, the GMT level in the serum of layers tested against anti-NDV, - HEV and -APV was influenced by age. Kowalczyk et al. (2019) also observed the effect of layers age on GMT anti-APV in serum. In our study, anti-APV GMT in females increased during the laying period, while in the study by Kowalczyk et al. (2019) in turkeys with a different vaccination schedule, the level of antibodies remained at a similar level throughout the laying period. However, when comparing the anti-NDV GMT in layers in our study to the results shown in another study (Kowalczyk et al. 2019), a higher level of anti-NDV was observed at the beginning of the laying period (36 weeks) and a lower one at the end (54 weeks), while in the 45th week of laying, the level of GMT anti-NDV in females was similar. Our study also observed that the serum GMT level in one-day-old chicks tested against anti-APV and -NDV varied significantly depending on the age of the laying layers. GMT anti-APV in the chick serum increased with the age of the females, while GMT -NDV decreased. Kowalczyk et al. (2019) also observed that with the age of layers, the level of GMT in the serum of chicks tested against -NDV also decreased. The same study also showed that the age of layers significantly influenced GMT in chick serum tested against *Ornithobacterium rhinortacheale*. In our study, the age of females had no effect on GMT in the serum of chicks tested against anti-HEV. Kowalczyk et al. (2019) also showed no effect of female age on GMT in chick serum tested against anti-APV and *Pasteurella multocida*. In a situation where chicks do not have MatAb, they should be vaccinated as soon as possible, and the immune response in this case proceeds normally since there is no MatAb interference directed against vaccine antigens (Suardana et al. 2023). On the other hand, a high level of MatAb may neutralize the vaccine virus, which may result in immunization failure, e.g. in the case of vaccination against infectious bursal disease (IBDV) (Gharaibeh et al. 2013). The level of MatAb in chicks, as mentioned above, is influenced by the transfer of antibodies from parents to offspring, which depends on the age of the females and the disease entity (Ali and Hasan 2018). The average transfer of APV and NDV antibodies from turkey to poult during the laying period was 51.7% and 37.6% in our study. In previously conducted studies by Kowalczyk et al. (2019), in which a different vaccination schedule was used, the percentage of NDV antibodies transmitted to offspring was higher (51.9%), while the transfer of APV was lower (33.2%) compared to the results in in our study during the entire reproductive period of turkey layers. In other studies, Ali and Hasan (2018) showed a decrease in the transfer of NDV and IBV antibodies and an increase in IBDV to offspring in broiler breeders during the reproductive period. However, other researchers Abdelhamid et al. (2024), showed that with the age of females (60-64 weeks), the transfer of specific NDV antibodies to offspring increased (14.13% - 19.35%). In another study performed on breeder layers, the average transfer of specific antibodies for IBV and NDV during the laying period was 30% (Hamal et al. 2006). Abdel-Moneim and Abdel-Gawad (2006) showed the average transfer of specific IBDV antibodies as 30-53% during the entire reproductive period of broiler breeders. However, Hamal et al. (2006) observed that regardless of the breeding line of birds, the average transfer of NDV and IBV antibodies was approximately 30% during the reproductive period.

Conclusions

A correlation between the level of serum antibodies (anti-NDV, -APV, and - HEV) in the parent flock and the level of MatAb in the offspring was shown. However the results vary depending on the diseases against which the breeder layers were vaccinated. The highest percentage vertical transfer to offspring was shown for anti-HEV antibodies, while the lowest was for anti-NDV antibodies. During the laying period, in the parent flock, the level of humoral immunity expressed by GMT towards anti-NDV decreased with the age of the females, while anti - APV and - HEV increased. All the above should be considered in preparing a vaccination schedule (both for breeder flocks and turkey chicks).

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