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Sex ratio of offspring in turkeys with different genotypes

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Abstract. Artificial regulation of the sex ratio in poultry is a critical component of intensive livestock production. It is obvious that the sex of a bird is determined not only by genetic factors but also by a number of other factors, which greatly complicates the introduction of any desired changes in the sex ratio and the solution of tasks that have significant scientific and industrial importance. The article presents results on the formation of androgenesis (male sex development) and gynogenesis (female sex development) in turkeys, due to genotype. The results demonstrate that the sex ratio in progeny significantly varies depending on parental genotype and genotype frequency. Specifically, turkeys with genotypes *Tf*CC *Cp*BC *Hb*BC and *Tf*CC *Cp* – *Hb*BC produced predominantly male offspring (78% and 69%, respectively). Conversely, genotypes *Tf*AA *Cp*CD *Hb*AB and *Tf*BD *Cp*CD *Hb*AA resulted in predominantly female offspring (65%). In contrast, turkeys with genotypes *Tf*AB *Cp*BD *Hb*CC, *Tf*BC *Cp*AB *Hb*BD, and *Tf*CD *Cp*CC *Hb*CD exhibited a balanced sex ratio in their offspring ($P < 0.05$). These findings highlight the potential use of genotype-based sex prediction as a genetic marker tool in turkey selection and breeding programs, offering a valuable approach for optimizing productivity in commercial poultry farming.

Key words: turkey, allele, androgenesis, gynogenesis, locus, polymorphic protein.

INTRODUCTION

Sexual reproduction is characteristic of both animals and plants and offers several advantages over asexual and vegetative reproduction (Otto, 2008; Biewer et al., 2015; Marron et al., 2025). A fundamental requirement for sexual reproduction is the presence of male and female sexes, as well as a specific quantitative ratio between them.

Sex is defined as a set of morphological and physiological characteristics of an organism that enable sexual reproduction through the formation of gametes and subsequent fertilization (Clinton, 1998; Donald Paul, 2007; Trukhina et al., 2015; Hirst

et al., 2018; Trukhina et al., 2018). In most animal species, including birds, the sex ratio at the population level is generally close to 1:1; however, deviations from this equilibrium may occur under the influence of various genetic and environmental factors (Collier et al., 2007). What mechanisms maintain this balanced sex ratio, and how can the regulation of sex be achieved? A definitive answer to these questions only became possible with the advancement of cytogenetic research and the ability to study the structure of the chromosomal apparatus within the cell (Piprek, 2020).

The avian Z and W sex chromosomes are not homologous to mammalian sex chromosomes, as they originated from different ancestral autosomes. Comparative analyses show partial homology between the avian Z chromosome and mammalian chromosome 9. Of 24 genes mapped to the bird Z chromosome, 17 have orthologs on chromosome 9. Conversely, the ornithine transcarbamylase (OTC) gene on mammalian chromosome 9 has an ortholog on the avian Z chromosome, indicating conserved synteny between these regions (Fridolfsson et al., 1998; Buchen, 2010; Ellegren, 2000; Kratochvíl et al., 2021).

The W chromosome in birds is thought to have evolved through inactivation and inversion of the Z chromosome. This hypothesis is supported by the presence of genes such as ACO1 and ZOV3, as well as the nucleotide sequence EEO6 (a microsatellite DNA marker), which are found on both the Z and W chromosomes of ratites. The avian W chromosome is characterized by low genetic diversity: approximately 60% of its DNA consists of tandem repeats of the Xho sequence (Mittwoch, 2004; Ezaz et al., 2006; Tsuda et al., 2007; Itoh et al., 2011; Peona et al., 2021).

In birds, unlike in mammals, females is the heterogametic sex (ZW), producing ova that carry either a Z or a W chromosome, while males are homogametic (ZZ) and produce sperm carring only the Z chromosome. Consequently, offspring sex is determined during oogenesis within the female, prior to fertilization. This system is known as the progammed (ZW) sex-determination mechanism (Lobashev, 1967; Matousek, 1971; Osterman, 1984; Magdy et al., 2022).

In natural populations, the sex ratio may deviate from 1:1 due to biological or environmental factors.

In poultry production, the genetic background of breeding lines and selection methods can significantly influence the expression of bioeconomic traits, often yielding inconsistent outcomes across different genotypes.

The scientific literature presents conflicting views regarding the influence of paternal and maternal inheritance on the determination of sex ratios in farm animals and poultry. Moreover, the results of existing studies often lack differentiated analysis, and the topic remains insufficiently explored, particularly in the context of Mendelian and polygenic traits (Lobashev, 1967).

According to current understanding, sex determination in birds is governed by two main factors: the number of Z chromosomes (ZZ develop as males, ZW as females) andthe presence of sex-determining genes on the W chromosome (Nakagawa, 2004; Smirnov & Trukhina, 2020). The balance theory of sex determination suggeststhat birds with a 3A:ZZZ karyotype are sterile males, while 3A:ZZW individuals develop as hermaphrodites with both an ovary and a left testis. Individuals with a 3A:ZWW karyotype form an ovary but typically die by day 16 of development. Gynandromorphs, characterized by left–right sexual mosaicism, are usually associated with ZZ or ZO karyotypes (Graves, 2003, 2013).

Molecular genetic studies have shown that both the Z and W chromosomes carry genes involved in sex determination. The CHD gene (chromodomain-helicase-DNA-binding protein), present on both the Z and W chromosomes as CHD-Z and CHD-W, plays a role in transcriptional regulation through changes in chromatin structure. However, this gene is absent on the W chromosome in ratites (e.g., ostrich, emu, and rhea) (Schmid et al., 2005).

The ASW gene (avian sex-specific, W-linked), located on the W chromosome (with the exception of keeled birds), is highly polymorphic, with more than 40 known copies. The activity or inactivity of this gene is believed to influence the development of sex-specific gonads (Rutkowska et al., 2012).

Further genetic studies of avian sex determination have identified key genes involved in gonadal differentiation, including FET1 gene (*female expressed transcript 1*) on the W chromosome (Loubser & Nikitina, 2025) and DMRT1 (*doublesex and mab-3 related transcription factor 1*) on the Z chromosome, which playing a significant role in development of the avian urogenital system and showing evolutionary similarity to genes found in humans, Drosophila, and nematodes (Fagegaltier & Baker, 2004; Sundström et al., 2004; Koopman, 2009; Matson et al., 2011).

In general, several genes have been implicated in the sex differentiation pathway in birds, including AMHSOX9, SOX3, WT1, SF1, and DAX1. These genes are involved during the embryonic stage of development, where they contribute to sex-specific physiological processes and the formation of reproductive organs (Sekido et al., 2004; Bagheri-Fam et al., 2010; Matsuda & Sakaizumi, 2016).

Despite these advances, the question of why the sex ratio of offspring varies depending on the maternal genotype remains unresolved. This phenomenon suggests a deeper interaction between maternal genetic background and sex-determination mechanisms, warranting further study.

To address this knowledge gap and to identify potential genetic markers for use in turkey breeding, a study was conducted for the first time in the Republic of Armenia to genotype turkeys at the transferrin (*Tf*), ceruloplasmin (*Cp*), and hemoglobin (*Hb*) loci in blood serum. They are well-studied polymorphic protein systems in birds, characterized by codominant inheritance and clear electrophoretic differentiation of genotypes, and therefore are widely used as informative genetic markers in population and breeding studies. At the same time, transferrin is involved in iron transport, ceruloplasmin in copper metabolism, and hemoglobin in oxygen transport, which indicates their important physiological significance. In addition, these loci showed a sufficient level of polymorphism in the studied turkey population, making them suitable for evaluating their possible association with offspring sex ratio (Grispo et al., 2012).

The experimental research also examined key bioeconomic traits in turkeys, including parthenogenesis, egg fertilization and hatchability rates, survival of young birds, live weight, growth rate, and others (Badalyan, 2010; Badalyan et al., 2021).

This paper presents the results of these experimental investigations, focusing specifically on the sex ratio of offspring produced from turkeys with different genotypes. The findings offer promising insight into the use of genotype-dependent sex ratio patterns as genetic markers for application in selection and breeding programs aimed at improving efficiency and productivity in turkey farming.

MATERIALS AND METHODS

Experimental studies were conducted at the subsidiary farm of Saakyan Shin CJSC in the Republic of Armenia. The research was carried out on 31 unrelated turkeys (*Meleagris gallopavo Linnaeus, 1758*) of the white broad-breasted breed, including 1 male (turkey cock). The turkeys were raised under standard floor-rearing conditions typical for the farm.

The experimental group was selected based on the principle of analogues, ensuring that all individuals were of the same age, sex, breed-specific coloration, exterior conformation, and body physical constitution. A combination of classical livestock methods, physiological-biochemical techniques, hybridological analysis, population-genetic approaches, and biometric methods were employed throughout the study.

The experimental group was selected according to the principle of analogues and included 18-month-old, sexually mature, clinically healthy birds of the same breed, characterized by breed-specific exterior conformation and constitution, a well-developed breast, strong limbs, normal mobility, and adequate body condition. This made it possible to form a homogeneous group and to minimize the influence of external factors on the research results.

Hatching of poults was conducted under natural incubation conditions. The sex of day-old turkey poults was determined using the Japanese method (Lukanov et al., 2018; Isa et al., 2020).

- Fertility was determined by the ratio of fertilized eggs to the total number of eggs set for incubation, expressed as a percentage.
- Hatchability was determined by the ratio of the number of chicks obtained to the total number of incubated eggs, expressed as a percentage.

Blood samples were collected from the axillary vein of turkeys using vacuum tubes with gel activator. Genotyping was performed at three polymorphic blood protein loci: transferrin (*Tf*), ceruloplasmin (*Cp*), and hemoglobin (*Hb*), using polyacrylamide gel electrophoresis.

The observed protein polymorphism was based on multiple allelism, and the inheritance of these proteins follows a codominant pattern. Thus, the phenotypic expression corresponds directly to the genotype, recorded as, for example, *Tf*AA, *Hb*BB, etc. (Matousek, 1971; Magdy et al., 2022).

Blood serum was obtained from blood samples collected without anticoagulant after complete clotting at room temperature, followed by centrifugation at 3,000 rpm for 10 min, after which the upper supernatant was carefully separated. Blood hemolysate was prepared after separation of the erythrocyte mass from plasma by washing it three times with 0.85% isotonic sodium chloride solution, followed by osmotic haemolysis with distilled water. The resulting homogenate was centrifuged again to remove membrane residues, and the supernatant was used as hemolysate for subsequent electrophoretic analysis.

Blood serum and hemolysate were prepared according to standard laboratory procedures (Grindem, 2011).

Electrophoretic analysis was conducted using the Davis method (Osterman, 1984) on a Multigel-Long electrophoresis system (Biometra, Germany), with 10% polyacrylamide gel (Table 1).

Table 1. Necessary conditions for electrophoresis of blood serum proteins (*Tf*, *Cp* and *Hb*) in experimental white broad-breasted turkeys (n = 31).

Proteins	Gel, %	Gel length, cm	Sample titer	Buffer		Voltage, V	Duration of phoresis, hour
				gel	electrode		
Tf	10	12	1:2	0.05 *M* tris-HCl, pH = 8.8	0.016 *M* tris - glycine, pH = 8.7	80	3.0
Cp	10	12	1:1	0. 18 *M* tris-HCl, pH = 8.8	0.016 *M* tris - borate, pH = 9.0	90	2.5
Hp	10	12	1:1	0., 2 *M* tris-citrate, pH = 8.8	0.0 8 *M* tris - borate, pH = 8.7	50	3.5

All laboratory-based genetic and biochemical analyses were performed in 2022 at the Biological Research Laboratory of Scientific Center of Agrobiotechnology of the National Agrarian University of Armenia.

The results obtained in 2022–2024 were processed using biometric methods (Merkuryeva, 1970) and subjected to statistical analysis employing a range of formulas, as well as the SPSS and Microsoft Excel 2010 software packages.

The frequency of genotypes and alleles was determined using the following formula:

$$P_i = \frac{n_i}{N}, \quad (1)$$

where P_i – frequency of an allele or genotype; n_i – the number of individuals carrying the allele; N – the total number of individuals studied.

The arithmetic mean was determined using the formula:

$$M = \frac{\sum V}{n}, \quad (2)$$

where M is the arithmetic mean; $\sum V$ – sum of all observations; n – number of observations.

The standard deviation was determined using the formula:

$$\sigma = \sqrt{\frac{\sum (V-M)^2}{n-1}}, \quad (3)$$

where σ – standard deviation.

The coefficient of variation was determined by the formula:

$$C_v = \frac{\sigma}{M} \cdot 100, \quad (4)$$

where C_v – coefficient of variation.

The error of the arithmetic mean was determined by the formula:

$$m_M = \frac{\sigma}{\sqrt{n}}, \quad (5)$$

where m_M – error of the arithmetic mean.

The reliability criterion of the arithmetic mean was determined by the formula:

$$t_M = \frac{M}{m_M}, \quad (6)$$

where t_M – reliability of the arithmetic mean.

The criterion for the reliability of the difference in parameters was determined by the formula:

$$t_D = \frac{D}{m_D}, \quad (7)$$

where t_D – reliability of the difference in parameters; D – the difference between maximum and minimum values of the variable feature; m_D – the difference in errors of the arithmetic mean.

The total variance was determined using the formula:

$$C_y = \frac{\sum(M_i - M_0)^2}{n}, \quad (8)$$

where C_y is the total variance; M_i – arithmetic mean of each group, M_0 – overall arithmetic mean of all groups, n – number of groups.

Means, standard errors, standard deviations, and coefficients of variation were calculated, while differences between groups were evaluated using the two-tailed Student's *t-test*. Statistical significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

The regulation of the sex ratio in agricultural poultry holds significant scientific and practical importance, as the optimal ratio of males to females depends on the intended direction of poultry production. For instance, male turkeys outperform females in terms of growth rate, feed efficiency, live weight, and slaughter yield by approximately 18–33%, offering substantial economic advantages. Conversely, in egg production systems, day-old male chicks are culled, as raising them further is economically unviable. Thus, artificial regulation of the sex ratio in birds becomes a critical aspect of modern poultry management.

Today, advanced genetic selection methods are increasingly employed in the breeding of farm animals and birds. These methods are based on polymorphic genetic systems, including blood group antigens, polymorphic proteins, and satellite DNA sequences. Among these, biochemical protein polymorphism has proven to be especially relevant. Numerous studies indicate that more than half of the proteins in farm animals and birds exhibit genetic polymorphism, meaning the encoding genes possess multiple alleles. Such proteins serve as biochemical markers of structural genes (Matousek, 1971; Magdy et al., 2022).

Table 2. Genetic structure of experimental white broad-breasted turkeys (n = 31) by *Tf, Cp* and *Hb* locuses: allele and genotype frequencies

Locuses	n	Frequency of occurrences, P_i								
		alleles			genotypes					
		A	B	C	AA	AB	BC	BD	CC	CD
Tf	31	0.28	0.33	0.39	0.12	0.16	0.21	0.12	0.19	0.20
Cp	31	0.38	0.27	0.35	0.18	0.20	0.11	0.16	0.23	0.12
Hb	31	0.24	0.39	0.37	0.11	0.13	0.18	0.21	0.18	0.19

Analysis of the electrophoretic profiles (phoregrams) presented in Table 2 revealed that the transferrin (*Tf*) locus in the experimental turkeys was polymorphic, comprising the alleles *Tf*A, *Tf*B, and *Tf*C with allele frequencies of 0.28, 0.33, and 0.39, respectively.

The *Tf*A allele of transferrin formed two genotypes: homozygous *Tf*AA (0.12) and heterozygous *Tf*AB (0.16). The *Tf*B allele produced two heterozygous genotypes: *Tf*BC (0.21) and *Tf*BD (0.12). The *Tf*C allele formed one homozygous genotype *Tf*CC (0.19) and one heterozygous genotype *Tf*CD (0.20).

The ceruloplasmin (*Cp*) locus also demonstrated polymorphism, comprising the alleles *Cp*A (0.38), *Cp*B (0.27), and *Cp*C (0.35). The *Cp*A allele formed two genotypes: homozygous *Cp*AA (0.18) and heterozygous *Cp*AB (0.20). Similarly, the *Cp*B allele produced two heterozygous genotypes: *Cp*BC (0.11) and *Cp*BD (0.16). The *Cp*C allele also followed this pattern, forming homozygous *Cp*CC (0.23) and heterozygous *Cp*CD (0.12) genotypes.

The hemoglobin (*Hb*) locus showed a similar degree of polymorphism, consisting of *Hb*A (0.24), *Hb*B (0.39), and *Hb*C (0.37). The *Hb*A allele formed *Hb*A (0.11) and *Hb*AB (0.13) genotypes. The *Hb*B allele was involved in the formation of *Hb*BC (0.18) and *Hb*BD (0.21) genotypes. The *Hb*C allele formed both homozygous *Hb*CC (0.18) and heterozygous *Hb*CD (0.19) genotypes.

Among the numerous possible combinations of genotypes, seven distinct composite genotypes were observed in the experimental turkey group.

1. *Tf*AA *Cp*CD *HbAB*,
2. *Tf*AB *Cp*BD *Hb*CC,
3. *Tf*BC *Cp*AB *Hb*BD,
4. *TfBD Cp*CD *Hb*AA,
5. *Tf*CC *Cp*BC *Hb*BC,
6. *Tf*CC *Cp– Hb*BC,
7. *Tf*CD *Cp*CC *Hb*CD.

The sex ratio of turkey poults obtained from hens with different genotypes**,** along with the results of selected bioeconomic traits, are presented in Table 3, while the outcomes of their biometric analysis are summarized in Table 4.

Analysis of the data presented in Table 3 shows that the overall sex ratio of the offspring in the entire experimental turkey flock (31 individuals) is approximately 1:1. However, the same table also reveals that the sex ratio varies significantly depending on the genotype of the hens and the frequency of occurrence of those genotypes, sometimes favoring either the male or female sex.

For example, turkeys with the genotypes *Tf*AB *Cp*BD *Hb*CC, *Tf*BC *Cp*AB *Hb*BD, and *Tf*CD *Cp*CC *Hb*CD produced offspring with an average sex ratio close to 1:1. Birds with these genotypes accounted for 54.8% of the total experimental group.

In contrast, interesting results were observed for turkeys with the genotype *Tf*CC *Cp*BC *Hb*BC, which produced 78% males and only 22% females. This genotype was present in 10% of the experimental group, with a frequency of 0.1. Similarly, in birds with the *Tf*CC *Cp– Hb*BC genotype, male offspring also predominated (69% males and 31% females), again at a frequency of 0.1.

Table 3. Sex ratio, fertility and hatchability of turkey-poults obtained from white broad-breasted turkeys (n = 31) with different genotypes

No of turkevs	Genotypes	Frequency of occurrences, P_i	Namber of hatched eggs, pcs.	Fertility, %	Hatchability, %	Sex of turkey-poults, %	
						♂	♀
1	*Tf*AA *Cp*CD *Hb*AB	0.13	23	93	85	33	67
2			20	91	83	33	67
3			24	93	81	39	61
4			24	92	83	37	63
5	*Tf*AB *Cp*BD *Hb*CC	0.16	22	91	84	47	53
6			23	91	83	52	48
7			23	92	85	53	47
8			22	90	85	44	56
9			20	94	85	50	50
10	*TfBC Cp*AB *Hb*BD	0.19	21	94	83	53	47
11			24	91	84	55	45
12			24	91	83	50	50
13			20	93	84	51	49
14			23	92	84	47	53
15			22	93	81	47	53
16	*Tf* BD *Cp*CD *Hb*AA	0.13	23	93	83	35	65
17			24	90	83	37	63
18			20	94	85	31	69
19			24	93	81	36	64
20	*Tf*CC *Cp*BC *Hb*BC	0.10	24	91	80	79	21
21			23	92	81	75	25
22			24	95	80	81	19
23	*Tf*CC *Cp – Hb*BC	0.10	23	93	82	70	30
24			24	91	81	69	31
25			22	94	83	69	3 1
26	*Tf*CD *Cp*CC *Hb*CD	0.19	21	91	81	54	46
27			24	90	83	50	50
28			23	91	82	47	53
29			24	93	81	44	56
30			24	94	80	50	50
31			21	93	83	47	59

A different trend was observed in the offspring of hens with the *Tf*AA *Cp*CD *Hb*AB genotype, where 64.5% were females and 35.5% were males. This genotype had a frequency of 13% in the experimental group. The genotype *Tf*BD *Cp*CD *Hb*AA, with the same frequency of occurrence (0.13), produced offspring in which 65.3% were females.

Table 4. Results of the analysis of biometric data

No.	Turkeys genotypes	Sex of turkey-poults	$M \pm m$	Standard deviation, σ	Variance, C	Coefficient of variation, C_V	Reliability of the arithmetic mean of sex ratio, t_m
1	*Tf*AA *Cp*CD	♀	64.5 ± 1.50	3.00	9.00	4.65	43.00
	*Hb*AB	♂	35.5 ± 1.50	3.00	9.00	8.45	23.66
2	*Tf*AB *Cp*BD	♀	50.8 ± 1.66	3.70	13.70	7.28	30.60
	*Hb*CC	♂	49.2 ± 1.66	3.70	13.70	7.52	29.63
3	*TfBC Cp*AB	♀	49.5 ± 1.31	3.21	10.30	6.48	37.78
	*Hb*BD	♂	50.5 ± 1.31	3.21	10.30	6.36	38.55
4	*Tf*BD *Cp*CD	♀	65.3 ± 1.31	2.63	6.92	4.03	49.43
	*Hb*AA	♂	34.8 ± 1.31	2.63	6.92	7.57	28.81
5	*Tf*CC *Cp*BC	♀	21.7 ± 1.76	3.06	9.33	14.1	12.33
	*Hb*BC	♂	78.3 ± 1.76	3.06	9.33	3.87	44.48
6	*Tf*CC *Cp*–	♀	30.7 ± 0.33	0.58	0.33	1.91	21.81
	*Hb*BC	♂	69.3 ± 0.33	0.58	0.33	0.89	21.00
7	*Tf*CD *Cp*CC	♀	51.3 ± 1.41	3.44	11.87	6.71	31.38
	*Hb*CD	♂	48.7 ± 1.41	3.44	11.87	2.98	34.54

Based on the numerical sex ratio of the resulting offspring, experimental mothers can be divided into three main groups:

1. *Tf*AA *Cp*CD *Hb*AB; *Tf*BD *Cp*CD *Hb*AA, when females predominate in the offspring (65%),

2. *Tf*CC *Cp*BC *Hb*BC; *Tf*CC *Cp*– *Hb*BC, when males predominate in the offspring (73.8%),

3. *Tf*AB *Cp*BD *Hb*CC; *Tf*BC *Cp*AB *Hb*BD; *Tf*CD *Cp*CC *Hb*CD, when males and females are present in equal numbers (Fig. 1).

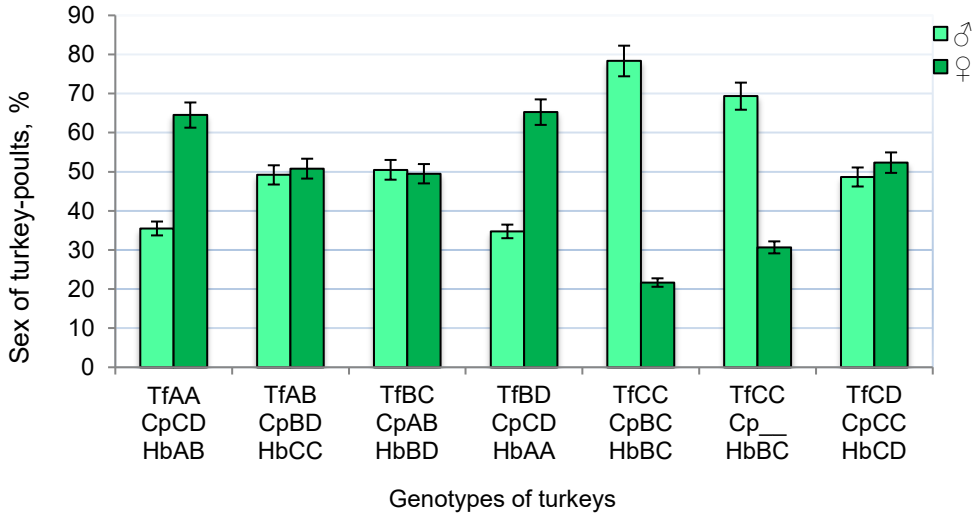


Figure 1. The sex ratio of turkey-poults obtained from white broad-breasted turkeys with different genotypes. Error bars correspond to standard deviation (SD). (On the X-axis - Genotypes of turkeys; on the Y-axis - Sex of turkey-poults, %)

Differences in the sex ratio between the indicated genotype groups were statistically significant ($P < 0.05$).

The analysis of the table data brings to the forefront a new question: by what mechanism is such a sex ratio regulated?

It is obvious that, in determining the sex ratio, primary importance is given to the parents' age, the quality of the gametes, physiological condition, the nature of the diet, breeding methods, and, of course, heredity (Kryeziu et al., 2018; Sapsuha et al., 2022). It follows that during oogenesis in experimental turkeys with different genotypes, unequal numbers of gametes or ova containing Z and W chromosomes were formed.

It is obvious that for turkeys – agricultural birds characterized by polygenic determination – the identification of genetic markers is highly relevant, since the prediction of the results of marker-assisted or genomic selection largely depends on the level of knowledge about the genetic structure of populations and the relationships between individual loci and alleles and economically valuable traits.

The emergence of sexual reproduction represents a crucial milestone in the evolution of living organisms, significantly enhancing genetic variability within populations and serving as an inexhaustible source of genetic material for natural selection. The pronounced expression of sexual dimorphism in freely reproducing natural populations is a direct outcome of natural selection (Otto, 2008).

Despite the widespread nature of sexual dimorphism, its role in progressive evolution remains insufficiently studied. Furthermore, domestication and the extensive use of artificial insemination have altered the natural male-to-female ratio in breeding populations, consequently reducing the influence of both sexes on the formation of the gene pool (Clinton, 1998).

Pre-identification of sex is crucial for developing effective breeding strategies in farm animals and poultry. It has significant implications for evolution, behavior, and physiology (Liu et al., 2025). However, modern breeding programs often fail to adequately consider sexual dimorphism. Long-term one-sided selection, focused primarily on economically valuable traits, has led to a decline in fertility rates among certain breeds of farm animals and poultry (van der Heide et al., 2016).

Among all agricultural birds, turkeys have received particular attention due to the significant difference in live weight between males and females. Consequently, regulating the numerical sex ratio is a pressing theoretical and practical challenge in poultry farming.

To address this issue, new genetic methods have been increasingly applied in the selection of farm animals and poultry. These methods rely on polymorphic genetic determination systems, which are considered genetically inherited traits. The biochemical diversity of proteins within these polymorphic systems has proven highly effective for genetic analysis due to its unique characteristics (Smiltina & Grislis, 2018).

It is evident that sex determination is governed by multiple genes, making its regulation a highly complex process. The primary objective of our research was to identify genetic markers associated with the dominance of one sex over the other, thereby contributing to a more precise and scientifically informed approach to sex ratio regulation in poultry breeding.

Based on the results of our research on the sex ratio of the offspring, the experimental turkeys can be grouped into three major categories:

1. Female-biased offspring:
   - *Genotypes*: *Tf*AA *Cp*CD *Hb*AB; *Tf*BD *Cp*CD *Hb*AA
   - *Sex ratio*: 64.5–65.3% females
2. Male-biased offspring:
   - *Genotypes*: *Tf*CC *Cp*BC *Hb*BC; *Tf*CC *Cp– Hb*BC
   - *Sex ratio*: 69–78% males
3. Balanced sex ratio (approximately 1:1):
   - *Genotypes*: *Tf*AB *Cp*BD *Hb*CC; *Tf*BC *Cp*AB *Hb*BD; *Tf*CD *Cp*CC *Hb*CD
   - *Sex ratio*: near 50% males and 50% females (Table 4).

Differences among the above genotype groups were statistically significant ($P < 0.05$).

When analyzing the data in Table 3, an important question arises: what mechanism regulates this sex ratio?

It is evident that several factors play a crucial role in determining the sex ratio, including the age of parental pairs, gamete quality, physiological condition, nutritional value, breeding methods, and, most importantly, heredity. These factors influence oogenesis, leading to an unequal distribution of Z- and W-bearing eggs in turkeys with different genotypes (Batra et al., 2022).

Thus, identifying genetic markers in turkeys is of significant importance, particularly given the polygenic nature of economically valuable traits in poultry. The ability to predict outcomes in marker-assisted or genomic selection depends largely on the availability of detailed genetic information, the population's genetic structure, and the relationships between specific loci, alleles, and key production traits.

CONCLUSIONS

Based on the analysis of the research findings, the following conclusions can be drawn:

1. The studied polymorphic blood protein loci (*Tf, Cp*, and *Hb*) in the experimental white broad-breasted turkeys were genetically variable and represented by several allelic forms, indicating the presence of biochemical genetic polymorphism in the examined population.

2. Seven combined genotype variants were identified among the 31 experimental turkey hens, demonstrating the genetic diversity of the studied group.

3. The sex ratio of offspring differed according to maternal genotype. In hens with genotypes *Tf*CC *Cp*BC *Hb*BC and *Tf*CC *Cp – Hb*BC, male offspring predominated, whereas in hens with genotypes *Tf*AA *Cp*CD *Hb*AB and *Tf*BD *Cp*CD *Hb*AA, female offspring predominated. In the remaining genotypes, the sex ratio was approximately 1:1.

4. The obtained data suggest that certain genotype combinations at the studied polymorphic protein loci may be associated with variation in offspring sex ratio in turkeys and may be of interest for further research and breeding programs.

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