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Uurlu, M; Akda, F; Teke, B; Salman, M

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Effects of Protein in Diet and Sex Ratio on Egg Production, Egg and Hatching Chick Weight, Fertility, Hatchability and Embryonal Mortality in Pheasants (Phasianus Colchicus)

Author(s)

Uğurlu M[I]
Akdağ F[I]
Teke B[I]
Salman M[II]

[I] Ondokuz Mayıs University Veterinary Faculty Department of Animal Breeding and Husbandry, 55220 Atakum, Samsun, Turkey
[II] Ondokuz Mayıs University Veterinary Faculty Department of Animal Nutrition and Nutritional Disease, 55220 Atakum, Samsun, Turkey

Mail Address

Corresponding author e-mail address
Mustafa Uğurlu
Faculty of Veterinary Medicine, Ondokuz Mayıs University, Kurupelit Campus, Atakum, Samsun, Turkey.
Tel: +90-362-3121919
Email: mustafaugurlu55@hotmail.com

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Embryonal mortality, hatchability, pheasant, protein levels, sex ratio.

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ABSTRACT

This study was performed to investigate the effects of crude protein (CP) in diet and sex ratio on egg production, egg and hatching chick weight and hatchability traits in pheasants (*Phasianus Colchicus*). In this study, the treatment groups were constructed as 15% CP-5:1; 15% CP-7:1; 18% CP-5:1; 18% CP-7:1 for CP and sex (male:female) ratio respectively. The eggs were obtained from 48 weeks old pheasant. In this study, it was determined that there was positive effective on egg production, egg and hatching weight and hatchability of fertile eggs (HFE) for 18% CP. In contrast, fertility (FR) ($p<0.05$) and hatchability rate of total eggs (HR) ($p>0.05$), EPEM ($p>0.05$) and MPEM ($p>0.05$) tended to decrease with increasing CP. It was found that there was egg production, egg ($p>0.05$) and hatching weight ($p>0.05$), FR ($p>0.05$), HR ($p>0.05$) and HFE ($p>0.05$) increased with increasing female for sex ratio. In terms of embryonic mortality, the EPEM ($p>0.05$) and LPEM ($p>0.05$) rate decreased with increasing female. For protein levels × sex ratio, it was found that hatchability traits were adversely affected for 15% CP-1:7. Also, it was determined that the lowest EPEM, MPEM and LPEM were determined for 7:1 in 18% CP group. Based on these results, to improve hatchability traits and a lower embryonic mortality rate, it may be beneficial to use 15% CP-5:1 or 18% CP-7:1 for pheasant breeding.

INTRODUCTION

In poultry breeding, high fertility, hatchability and low embriyonic mortality are the main goal for profitability and productivity. Fertility, hatchability and embryonic mortality can be influenced by egg weight (Sczerbinska *et al.*, 1999, Petek *et al.*, 2003, Abiola *et al.*, 2008, Caglayan *et al.*, 2009), lenght of storage period (Fasenko *et al.*, 2001, Tona *et al.*, 2004, Oral Toplu *et al.*, 2007), age of flock (Elibol *et al.*, 2002,Seker *et al.*, 2004, Sari *et al.*, 2010), breeding type (Ozbey & Esen 2007),composition of hen diet (Cufadar *et al.*, 2010, Felipe *et al.*, 2010, Mohiti-Asli *et al.*, 2012, Praes *et al.*, 2014) and sex ratio (Ipek *et al.*, 2004, Alsobayel & Albadry 2012, Narinc *et al.*, 2013) in poultry species.

The composition of hen diet has an effective factor on egg production (Khajali *et al.*, 2008; Perez-Bonilla *et al.*, 2012), egg weight (Whitehead *et al.*, 1991, Zimmerman 1997; Shim *et al.*, 2013) and hatching characters (Danicke *et al.*, 2000) in poultry.

The protein level of hen diet is one of the factors influencing egg production and egg weight. Some researchers reported the general tendency increase egg production and egg weight with the increase of protein levels in the diets (Gunawardana *et al.*, 2008; King'ori *et al.*, 2010; Mohiti-Asli *et al.*, 2012; Shim *et al.*, 2013). Some other researchers reported that egg production and egg weight was not affected by feeding low crude protein (Cho *et al.*, 2004; Khajali *et al.*, 2008). In poultry breeding, it is known that energy levels of the diet is important as well as protein levels of the diet. Leeson & Caston (1996) reported that the egg weight decreased when dietary protein content was reduced from 16.80% to 14.40% in isoenergetic hen diets. Li *et al.,* (2013) reported ranges of estimated optimum ME and CP intake were 2591 to 2683 kcal/kg and 15.58% to 16.64% CP for optimizing egg production of Lohmann Brown laying hens. Also, it was reported that diets with high protein and low energy have a negative effect on egg weight and egg production (Li *et al.,* 2013; Steenhuisen & Gous, 2016).

It was reported that there is a positive relationship between egg weight and hatching weight (Özcan *et al.*, 2001; Tona *et al.*, 2004). Therefore, the relationship between egg weight and protein levels of hen diet should be taken into consideration for hatching chick weight. Spratt & Leeson (1987) reported that a reduction in egg weight and consequently in chick weight decrease when dietary protein content was reduced from 16.70% to 12.70% for broiler breeder hens.

The protein levels of breeder diet is one important factor for hatching characters and embryonic mortality. A study with chukar partridge reported the general tendency increase infertility with high protein levels (CP 17%), and in contrary hatchability decreased under the same condition (Cufadar *et al.,* 2010). However, Mohiti-Asli *et al.*, (2012) found that fertility, hatchability of total and fertile eggs, and embryonic mortaliy of hen diet with high protein (CP 17.40%) were higher than those of hen diet with low protein (CP 14.50%). Some researchers reported that low crude protein (CP 12%) was not effective on fertility (Alsobayel, 1992) and hatchability (King'ori *et al.,* 2010) for local strains.

Sex ratio (male:female) is an important factor for fertility and hatchability. Generally, fertility tends to decrease with increasing sex ratio. Alsobayel & Albadry (2012) reported that sex ratio is efficient on fertility and hatchability of total eggs but not fertile hatchability and embryonal mortality in laying hens. Most researchers reported fertilization and hatching characteristics to decrease with increasing male: female ratio (Deeming & Wadland, 2002; Seker *et al.,* 2004; Narinc *et al.,* 2013). It was recommended that mating ratio of 1:1 and 1:2 produced the highest fertility and hatching characters in japanese quail (Seker *et al.,* 2004; Narinc *et al.,* 2013). Deeming & Wadland (2002) reported that a mating ratio of 8:1 produced a higher rate of egg production, fertility and hatchability compared with a 12:1 ratio.

As in the breeding of all poultry species, the effects of egg weight and lenght of storage period (Kirikci *et al.,* 2005, Demirel & Kirikci, 2009, Caglayan *et al.,* 2010), age of flock (Esen *et al.,* 2010; Ozbey *et al.,* 2011) and breeding type (Kirikci *et al.,* 2003; Genc & Ozbey, 2013) on successful hatching are among the most important influences for pheasants. However, there is little published work concerning the effect of protein levels (Monetti *et al.*, 1981) and sex ratio (Deeming & Wadland, 2002) on fertility, hatchability and embryonic mortality.

The National Research Council (NRC, 1994) reported the dietary metabolizable energy and crude protein requirements for breeder pheasants as 2800 kcal/kg ME and 15% CP, also other studies (Rhône Poulenc, 1993) recommended as 2900 kcal/kg ME and 17% CP for breeder pheasant and partridge. Therefore, it is important to differentiate the extent to which changes in hatchability traits are due to changes in protein levels of diet and sex ratio in pheasant.

As a contribution to efforts to increase the efficiency of pheasant breeding, the current study aimed to investigate the effects of the protein levels in the diets and sex ratio on hatchability and embryonal mortality.

MATERIAL AND METHODS

Animals, housing and designation of groups

A total of 360 eggs laid by 48 weeks old pheasant were obtained from the Samsun Gelemen Pheasant Breeding Centre of the Forest and Water Ministry of Turkey. In this study, the nutrition groups were classified as 15% CP and 18%CP. Sex ratio groups were designed as 5:1 and 7:1. After these classifications, a total of 4 groups were constructed, with each nutrition group divided into 2 subgroups for sex ratio. The composition of breeder diet was prepared according to the National Research Council (NRC, 1994). Formulation of breeder diet was performed by using to Microsoft Office Excel in the department of nutrition and nutritional disease. The chemical composition (protein, fat, dry matter and ash) of the experimental basal diets were analyzed according to the Association of Official Analytical Chemists (AOAC, 2000). Ingredient and chemical composition of experimental basal diets were presented in Table 1.The ration was applied on the breeding pheasants during the 4 weeks before laying period. The eggs were collected from small breeding flocks having 1:5 and 1:7 kept in open cages of 4 m × 5 m. The collected pheasant eggs were placed in trays and kept at 18 ˚C for 7 days. The hatching weights of the eggs were determined by measuring the eggs of all groups one by one in electronic scales delicate to 5g before hatching.

Table 1 – Ingredient and chemical composition of experimental basal diets.

Ingredients (%)	Protein levels	
	15% CP	18% CP
Corn	52.70	46.40
Wheat	19.50	13.50
Soybean (44% CP)	15.00	23.40
Sunflower meal (28% CP)	4.90	7.00
Vegetable oil	0.10	2.10
Dicalcium phosphat	1.80	1.71
DL-methionin	0.08	-
Calcium carbonat	5.25	5.25
Cocsidiostat	0.03	0.05
Antioxidant	0.04	0.04
L-lizin hidroklorid	0.03	-
Salt	0.30	0.30
Vitamin-Mineral	0.27	0.25
Energy:protein	180.30	153.42
Analyzed value		
Dry matter %	89.60	90.01
Crude protein %	15.49	18.27
Crude fiber %	5.00	5.08
Ether extract %	3.90	5.00
Total ash %	10.18	9.55
Calculated values		
Metabolizable energy, kcal/kg	2793	2803
Calcium %	2.50	2.46
Available phosphorus %	0.40	0.38
Lysine %	0.68	0.71
Methionine+cysteine %	0.59	0.60

CP: Crude Protein

Hatchery conditions, hatching chick weight, evaluation of incubation results

The selected pheasant eggs were placed in trays and kept at 18 ˚C and 75% moisture for 7 days, based on their collection days, and then transferred to a cupboard type incubator. The eggs were incubated at 37.7°C and 65% moisture for 21 days in the development section, and at 37.5°C and 90% moisture for the last 3 days (Cetin & Kirikci, 2000). At the end of the 24th day of the incubation period, with monitoring of the hatchings, hatching weights of the chicks determined by measuring the chicks of all groups, one by one in electronic scales delicate to 5g when the chick's bodies dried up after the hatching. Also, the eggs which had not hatched were broken one by one and observed with the naked eye. In that macroscopic examination, the stage of embryo development at death was classified in terms of 3 possible death periods. The classification was done as follows; EPEM: early period embryonic mortality (embryo developed, filling the eggshell, eyes developed); MPEM: middle period embryonic mortality (feathers developed, more of yolk sack external to the body) and LPEM: late period embryonic mortality (2/3 or whole of yolk sack in the body of embryo) (Akıncı *et al.*, 2000).

FR, HR and HFE were calculated as the fertile eggs / total eggs, chick number of hatched / total eggs and chick number of hatched / fertile eggs respectively. Also, EPEM, MPEM and LPEM were calculated as early period embryo mortality / fertile eggs, middle period embryo mortality / fertile eggs and late period embryo mortality / fertile eggs respectively (Aksoy, 1994).

Statistical Analysis

Least square variance analysis was performed for the comparision of egg and hatching weight in the different protein levels, sex ratio and protein levels × sex ratio groups, and determination of the significance of differences between the groups was done with the Duncan test. The Chi-square test was used for the comparision of FR, HR, HFE, EPEM, MPEM and LPEM values for the different protein levels and sex ratio groups (SPSS 1993).

RESULTS

Egg weight and hatching chick weight

The means of egg and hatching chick weight for protein levels and sex ratio groups are presented in Table 2. The egg and hatching chick weight for 15% CP group were lower than those of 18% CP. In respect of sex ratio, the egg and hatching chick weight for 5:1 group were lower than those of 7:1. Differences among egg weights ($p<0.01$) and hatching chick weight ($p<0.001$) for protein levels were significant while they were not significant for sex ratio ($p>0.05$) groups. For the diet of protein levels × sex ratio groups, it was determined that the highest egg weight was for 7:1 in 18% CP, but the highest hatching chick weight

Table 2 – Egg and hatching chick weight by groups (Mean ± S.E.)

Groups	Egg weight(gr)	n	Hatching weight(gr)	n
Protein levels	**		***	
15 %	32.73±0.16	178	22.32±0.17	128
18 %	33.43±0.15	182	23.22±0.17	129
Sex ratio	NS		NS	
5:1	32.87±0.16	173	22.59±0.17	127
7:1	33.29±0.15	187	22.98±0.17	130
Protein levels × sex ratio				
15 %-5:1	32.80±0.23	85	21.92±0.24	66
15 %-7:1	32.65±0.22	93	22.72±0.24	62
18 %-5:1	32.93±0.22	88	23.25±0.24	61
18 %-7:1	33.92±0.22	94	23.19±0.23	68

**: p<0.01, ***:p<0.001, NS: not significant was 5:1 in 18% CP. In this result, it was determined that protein levels were effective on egg and hatching chick weight but, sex ratio were not effective on them.

Egg production, fertility and hatchability

The mean values for egg production, fertility and hatchability of pheasant eggs for feeding system, sex ratio and diet of protein level × sex ratio interaction are given in Table 3. Egg production was determined as 427 and 450 eggs for 15% CP and 18% CP respectively. It was observed that there was egg production increased with increasing protein level. The highest FR ($p<0.05$) and HR ($p>0.05$) were determined for the 15% CP. However, the highest HFE ($p>0.05$) were for the 18% CP.

In terms of sex ratio, egg production was found as 430 and 447 eggs for 5:1 and 7:1 respectively. It was determined that there was egg production increased with increasing female. The FR, HR and HFE for 5:1 group were determined higher than those of 7:1 group but, there were no significant differences for FR ($p>0.05$), HR ($p>0.05$) and HFE ($p>0.05$).

For the diet of protein levels × sex ratio groups, it was determined that egg production increased with increasing protein level and sex ratio. The highest FR and HR were determined for 5:1 group in 15% CP whereas the highest HFE was determined for 7:1 group in 18% CP group.

Embryonic mortality

The mean values of embryonic mortality for protein levels in diet, sex ratio and protein level in diet × sex ratio interaction are given in Table 4. The total embryonal mortality rate for 15% CP group (16.84%) higher than that of 18% crude protein (10.42%). In the present study, embryonal mortality decreased with increasing CP for EPEM ($p>0.05$) and MPEM ($p>0.05$) whereas embryonal mortality increased for LPEM ($p>0.05$).

In terms of sex ratio, the EPEM ($p>0.05$) and LPEM ($p>0.05$) rate for 5:1 group higher than those of 7:1 group, whereas the highest MPEM ($p>0.05$) was for 7:1 group.

For interaction groups, it was determined that the lowest EPEM, MPEM and LPEM was determined for 7:1 in 18% CP group.

DISCUSSION

Egg weight and hatching chick weight

Many researchers reported that there were various effects in protein levels of hen diet on the egg weight in poultry species (Cho *et al.,* 2004; Khajali *et al.,* 2008; Gunawardana *et al.,* 2008; Kingori *et al.,* 2010; Shim *et al.,* 2013). Also, it was reported that there is a positive relationship between egg weight and hatching chick weight (Ozcan *et al.*, 2001; Tona *et al.*, 2004). Therefore, the relationship between egg weight and protein levels in hen diet is important for hatching chick weight.

In this study, mean egg weight and hatching chick weight was 32.73 and 22.32 g for 15% CP; 33.43 and 23.22 g for 18% CP, 32.87 and 22.59 g for 5:1; 33.29 and 22.98 g for 7:1 sex ratio respectively (Table 2). In the present study, mean egg weights were similar to those reported by Krystianiak *et al.*, (2005) namely, 31.33 g, and by Kozuszek *et al.*, (2009) namely 32.94 g respectively. When based on the 15% CP egg weight and hatching chick weight, increasing of egg weight and hatching chick weight were determined as 2.13% and 4.03% parallel to increasing crude protein percentage in this study respectively (Table 2). Likewise, many researchers reported the positive effects of dietary protein on egg weight (Gunawardana *et al.,*

Table 3 – Mean hatchability traits for protein levels, sex ratio and protein levels × sex ratio of pheasant eggs

Protein levels	Egg production(n)	Total eggs (n)	Fertile eggs (n)	Chick number (n)	FR (%)	HR (%)	HFE (%)
15 %	427	178	158	128	88.76	71.91	81.01
18 %	450	182	148	129	81.31	70.87	87.16
Chi-square					3.912*	0.047NS	2.149NS
Sex ratio							
5:1	430	173	150	127	86.70	73.41	86.70
7:1	447	187	156	130	83.42	69.51	83.42
Chi-square					0.760NS	0.666NS	0.751NS
15 %-5:1	211	85	73	66	90.68	77.60	85.70
15 %-7:1	216	93	85	62	87.10	66.70	76.60
18 %-5:1	219	88	73	61	82.95	69.31	83.56
18 %-7:1	231	94	75	68	79.78	72.34	90.66

FR: Fertility, HR: Hatchability Rate, HFE: Hatchability of Fertile Eggs

*:$p<0.05$, NS: not significant

2008; King'ori *et al.,* 2010; Shim *et al.,* 2013). Also, Spratt & Leeson (1987) reported that an increase in egg weight and consequently in chick weight increased when protein content in isoenergetic diet was changed from 12.70% to 16.70% for broiler breeder hens. The results of this studies indicated that egg and hatching chick weight increased with increasing crude protein in isoenergetic diet.

In laying poultry, there is scarce information about the effects of sex ratio on egg and hatching chick weight. In this study, it was found that there was increase in egg and hatching weight when mating ratio was changed from 1:5 to 1:7 for pheasant. Namely, egg and hatching weight for 7:1 were determined 1.28%, 1.73% higher than those of 5:1 respectively (Table 2). Likewise, a study with silver pheasant (Lophura nychemera) reported that an increase in male-female ratio from 1:2 to 1:5 mating ratio showed a positive effect on egg weight (Kaleem *et al.*, 2015). However, these results are lacking explanation for this effect.

For the protein levels of diet × sex ratio groups, some studies reported that diet with high protein and adequate energy had positive effect on egg weight and hatching chick weight (Gunawardana *et al.*, 2008; King'ori *et al.*, 2010; Shim *et al.*, 2013). However, some other studies found that egg weight was not affected by feeding low crude protein diets (Cho *et al.*, 2004; Khajali *et al.*, 2008). These conflicting results may be attributed to the different management and feeding conditions. Leeson & Caston (1996) reported that egg weight increased when dietary protein was increased in isoenergetic hen diets. Also, it was reported that diets with high protein and low energy have negative effect on egg weight. In this study, the highest egg weight and hatching chick weight was determined 18% CP for both of 1:5 and 1:7 sex ratio (Table 2). This result supports that protein levels were effective on egg and hatching weight but, sex ratio was no effective on them.

Egg production, fertility, hatchability and embryonal mortality

In poultry breeding, it has been reported that diet of protein and energy levels are effective factors on egg production (Li *et al.*, 2013; Steenhuisen & Gous, 2016). Some studies reported that egg production increased when dietary protein content was increased (Leeson & Caston, 1996; Praes *et al.*, 2014), also another study reported that diets with low protein exhibite negative effect on egg production (Keshawarz 1998; Cufadar *et al.*, 2010) in isoenergetic hen diets. However, it was reported that diets with high protein and low energy have a negative effect on egg production (Li *et al.*, 2013; Steenhuisen & Gous, 2016). In this study, hen diets with different protein levels (15%; 18%) and isoenergetic (2800 kcal/kg) were used. It was determined 5.38% increase in egg production parallel to increasing protein level in this study. Therefore, increasing egg production might be releated to the protein level of the diet in this study.

The sex ratio is recommended for commercial pheasant range from 1:7, 1:10 or 1:12 (Deeming & Wadland, 2002). The sex ratio designated in this study were 1:5 and 1:7. In this study, egg production was found as 430 and 447 eggs for 5:1 and 7:1 respectively. It was observed that egg production for 5:1 was lower than that for1:7 in this study. The low egg production of 5:1 could be due to decreasing of their female number.

For the diet of protein levels × sex ratio groups, in this study, it was determined that egg production increased with increasing protein levels for both protein levels and sex ratio. The highest egg production was found in 18%-7:1 group. It is well known that hen diets with high protein and adequate energy have positive effects on egg production (Leeson & Caston 1996; Praes *et al.*, 2014). It was reported that the egg production increased with female increasing (Deeming & Wadland 2002). This results supported that protein levels of diet and sex ratio had a synergistic effect on egg production.

In the present study, it was determined that the highest FR and HR was determined for 15% CP while the lowest HFE was determined for 15% CP (Table 3). Moreover, the total embryonal mortality rate for 15% CP (16.84%) was higher than that of 18% CP (10.42%) (Table 4). Especially, it was determined that MPEM for 15% CP was higher than that of 18% CP in this study. Supporting this finding, a study with pheasants reported that higher fertility and hatchability was determined in pheasants fed with ration with the poorest protein content (Monetti, 1981). Likewise, Cufadar & Bahtiyarca (2006) reported that diets with low protein (13%), conventional (17%) and higher protein (20%) had no different effect on fertility and hatching characters of breeder chukar partridge. However, another study with chukar partridge reported the general tendency increase infertility with high protein levels (CP 17%), in contrary hatchability decreased under the same condition (Cufadar *et al.*, 2010).

In this study, when the FR, HR and HFE were evaluated for sex ratio, the highest FR, HR and HFE were determined for 5:1 group in this study. Narinc *et al.*, (2013) reported as up to 3 female per male ratio for optimum fertility ratio for japan quails.

Table 4 – Embryonic mortality mean rates for protein levels, sex ratio and protein levels × sex ratio of pheasant eggs

Protein levels	EPEM	MPEM	LPEM
15 %	5.61	7.86	3.37
18 %	2.19	3.29	4.94
Chi-square	2.816^{NS}	3.580^{NS}	0.556^{NS}
Sex ratio			
5:1	4.62	3.46	5.20
7:1	3.20	7.48	3.20
Chi-square	0.488^{NS}	2.766^{NS}	0.895^{NS}
Protein levels × sex ratio			
15 %-5:1	7.05	3.52	2.35
15 %-7:1	4.30	11.82	4.30
18 %-5:1	2.27	3.40	7.95
18 %-7:1	2.12	3.19	2.12

EPEM: Early Period Embryonic Mortality, MPEM: Middle Period Embryonic Mortality, LPEM: Late Period Embryonic Mortality. NS: Non-significant

In this study, when the FR, HR and HFE were evaluated for sex ratio, the highest FR, HR and HFE were determined for 5:1 group in this study. Narinc *et al.*, (2013) reported as up to 3 female per male ratio for optimum fertility ratio for japan quails.

Female birds have larger number of males recruiting and decreasing mating ratio, therefore FR can be increased with decreasing for female:male. It was reported that the FR, HR and HFE for 8:1washigher than those of 12:1 for pheasants (Deeming & Wadland 2002). Alsobayel & Albadry (2012) reported from 1:6 to 1:14 changed mating ratio hatchability of fertile eggs, early embryonal mortality late embryonal mortality and total embronal mortality decreasing for Baladi Chickens. In the present sudy, the HR and HFE for 5:1 were determined as 5.31% and 3.93% higher than those of 7:1 respectively. The total embryonic mortality was determined as 13.28% and 13.88% for 5:1 and 7:1 respectively (Table 4). However, the MPEM (7.48%) rates was determined for the 1:7. Therefore, the loss of HR and HFE for 7:1 were attributable to an increase in MPEM.

For the protein levels of diets× sex ratio groups, the highest FR and HR was determined for15% CP and 1:5 sex ratio group while the highest HFE was determined for 18% CP and 1:7 (Table 3). The lowest embriyonal mortality was determined for 18% CP and 1:7 sex ratio group (Table 4). These results show that hatchability in both of protein levels and sex ratio is being determined by rates of fertility rather than embryonic mortality.

Consequently, in terms of protein levels of the diet, the FR and HR for 15% CP was higher than those of 18% CP. In contrast, HFE for 15% CP tended to decrease due to increasing total embryonic mortality. In this study, in terms of sex ratio, the FR, HR and HFE for 5:1 group was higher than those of 7:1. Total embryonic mortality had almost the same value for both sex ratios. However, it was observed that embryonic mortality for 7:1 was lower, except for MPEM. Based on these results, to achieve a higher hatchability rate and hatchability of fertile eggs, and a lower embryonic mortality rate, it may be beneficial to use 15% CP-5:1 or 18%CP-7:1 for pheasant breeding.

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