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Hatching Weight and Development of Metabolically Active Organs of Broiler Chicks Obtained from Carbohydrate Injected-Eggs

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Abstract:*Two hundred fifty fertile eggs from Ross 308 breeder (33-week-old) were allocated randomly to five groups with five replicates. Except the negative control group eggs (non injected; NC), 1 ml isotonic solution was injected into the positive control (PC) group eggs while the other groups were injected with 0.25 mg glucose (G), sucrose (S) and starch (CS) dissolved in 100 ml isotonic solution, respectively. The G had higher hatching weight (P<0.05) and relative yolk weight (P<0.05) compared to other treatments at hatch. The relative liver weight was higher in S and CS chicks than in G and NC chicks (P<0.05). The relative proventriculus weight of S and CS chicks were higher than G and PC chicks, while gizzard weight was higher than G chicks (P<0.05). In ovo CS nutrition increased the relative weights of duodenum and jejenum according to NC and G treatments, respectively (P<0.05). Injection of S increased total digestive system (P<0.05). Although the impacts of in ovo different carbohydrates nutrition on whole digestive system or different parts of GIT were not at the same direction and stability our results shows that the in ovo carbohydrate nutrition lead to more developed digestive system and heavier chicks.*

Keywords*:In ovo, glucose, sucrose, corn starch, digestive system, yolk sac.*

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I. Introduction

In commercial poultry industry, chicks meet the nutrient requirements from the fat and protein contained in the yolk sac (YS) until all have been placed to hen (approximately 36-48 hours) (Noy and Sklan, 1999; Sklan and Noy, 2000). However, depending on the genotype, the egg nutrients may be insufficient for the complete development of embryonic tissues (Grodzik et al., 2013). This may lead to inadequate development or damage of metabolically active organs and tissues in the perinatal period, resulting with increased weight loss (Noy and Sklan, 1999; Bhuiyan et al., 2011) and even death rate (Willemsen et al., 2010). In this period, physiological and metabolic changes occur that may significantly affect embryonic survival and subsequent performance (Collin et al., 2007; Leksrisompong et al., 2007). These changes in poultry embryos together with the energy metabolism of the embryo and the excessive use of nutrients from amniotic fluid and tissue protein reserves to store glycogen in muscle and liver (Keirs et al., 2002). The use of gluconeogenesis of proteins in residual YS or embryonic tissues may lead to a decrease in hatching weight and consequently decrease in life force and growth performance. In order to overcome these problems, there has been an increasing awareness of the *in ovo* nutrition with individual or combination of carbohydrates, proteins and amino acids (Uni et al., 2005; Abdulqader et al., 2017).

In ovo carbohydrate feeding (maltose, sucrose, dextrin)promotes intestinal development by increasing disaccharide digestion in perinatal period (Tako et al., 2004; Smirnov et al., 2006). Uni et al. (2005) reported that *in ovo* carbohydrate (maltose, sucrose, dextrin) and beta-hydroxy methyl butyrate (HMB) feeding improves the energy status and early growth of the late-stage broiler chick embryo. Bhanja et al. (2008) determined that *in ovo* glucose injection increases the relative weights of some organs such as liver, gizzard, proventriculus and small intestine. Bottje et al. (2010, turkey) and Tangara et al. (2010, duck) reported that *in ovo* dextrin, sucrose, maltose and arginine blends provide the energy for perinatal growth by increasing glycogen reserves of the embryo and ultimately increased the hatchability and hatching weight of poults. Ebrahimnezhad et al. (2011) and Zhai et al. (2011a) determined that respectively *in ovo* glucose and *in ovo* glucose, fructose, sucrose, maltose and dextrin injection decrease hatchability while increase hatching weight in broilers. Similarly,

Salmanzadeh et al. (2012) reported that *in ovo* glucose, magnesium and glucose + magnesium feeding decreased hatchability but glucose and glucose + magnesium injection increased breast muscle size and performance.

Poultry can use starch from polysaccharides, saccharose and maltose from disaccharides and glucose, fructose, mannose and galactose from monosaccharides as a source of energy(Özen, 1986). The use of these carbohydrates in *in ovo* feeding can reduce the use of amino acids in glycogenogenesis (Kornasio et al., 2011) and thus increase the growth of metabolically active organs and tissues, and hence the chick hatching weight. Protein synthesis can be supported in muscles. However, to our knowledge, there is no published study comparing the use and activities of monosaccharides, disaccharides and polysaccharides in *in ovo* feeding. Therefore, the aim of the present study was to determine the effect of the *in ovo* glucose, saccharose, and corn starch injection which are monosaccharides, disaccharides and polysaccharides, on the hatchability, hatching weight, yolc sac weight and digestive system development and liver color values of chicks at hatch.

II. Materials and methods

A total of 250 Ross308 fertile eggs from Ross308 breeder (30-week-old) were randomly allocated to five groups with five replicates of 10 eggs each with an average egg weights and were incubated under routine conditions (37.8 C and 60% RH) at Eskişehir Osmangazi University Faculty of Agriculture Research and Application Farm. On the 18th day of the incubation, except the negative control group eggs (non injected; NC), 1 ml isotonic solution was injected into the positive control (PC) group eggs while the other groups were injected with 0.25 mg glucose (G), sucrose (S) and starch (CS) dissolved in 100 ml isotonic solution, respectively. Eggs were injected with 1 ml solution 27-gauge needle into amnion as reported by Uni et al. (2005). The eggs of the NC group were also treated in a similar process as the *in ovo*-injected eggs with the exception of injection.

In order to calculate the hatchability, live chicks obtained from each tray were counted and calculated as a percentage of the number of fertile eggs set. Upon hatch after all chicks were weighted two chicks from each replication with body weight within 1 standard deviation of the mean treatment weight (10 chicks per treatment) were slaughtered to determine weights of yolk sac, gizzard, proventriculus, heart, livers and weights and lenghts of whole GIT, duodenum, jejunum and ileum (Tabeidian et al., 2011). Relative weight (g / 100 g CA) and lengths (cm / 100 g CA) of these parameters were calculated by percantage the absolute weight (g) and length (cm) to the live weight.

Because the liver color characteristics are indicative of fatty acid and glycogen storage status (Northcutt et al., 1997; Ocak and Sivri, 2008), color characteristics of liver (L*, a*, and b*) were performed with Minolta CR 300 Chroma Meter (Minolta Camera Co., Osaka, Japan) which was calibrated with spectrocolorimeter white color plate Minolta calibration plate, No. 21733001, Y=92.6, x=0.3136, y=0.3196). The measurements were made from at least two different points of the liver, depending on the size of the liver. CIE standards (CIE L * = brightness, a * = redness and b * = yellowness values) were applied (D65, 10°) to liver color measurements and three basic color characteristics (L * 100 = white, 0 = black, red color coordinate, a * ± red - green and yellow color coordinate b * ± yellow - blue) were noted (CIE, 1986). All animal procedures were approved by the local Ethical Committee of Eskisehir Osmangazi University for Experimental Animals.

The data obtained from this study were analyzed in SPSS 17.0 package programme. Kolmogorov-Simirnov test was applied for the assumption of normality of the data and homogeneity of the variances was evaluated by Levene test. The data of the study were subjected to Anova Analysis and Duncan multiple comparison test was used to determine the differences among the means.

III. Results and Discussion

Hatchability was not affected by *in ovo* different carbohydrate injection but compared to other treatments, the chicks obtained from the G injected eggs had higher hatching weight (Table 1, $P < 0.05$) and yolk sac weight (Table 2, $P < 0.05$). Relative liver weight was higher in S and CS chicks than in G and NC chicks ($P < 0.05$). Relative proventriculus weight of S and CS chicks was higher than that of G and PC chicks, and the gizzard weight was higher than that of Gchicks ($P < 0.05$).*In ovo* CS feeding increased relative duodenum and jejenum weights compared to NC and G treatments, respectively ($P < 0.05$). Sucrose injection increased total digestive system weight compared to G and NC treatments ($P < 0.05$). Liver weight was higher in S and CS chicks than in G and NC groups (Table 3, $P < 0.05$) but there was no difference between the treatments in terms of liver color values ($P > 0.05$).

Table (1): Hatching characteristics of chick obtained from eggs without injection (NC), *in ovo* saline (PC), glucose (G), sucrose (S) and corn starch (CS) injected

	NC	PC	G	S	CS	P	SEM
Egg weight, g	65.80	65.68	65.36	65.68	65.80	0.393	0.078
Hatching weight, g	47.91^{b}	47.30^{b}	49.69^{a}	47.70^{b}	45.96^{b}	0.046	0.424
EW/HW	1.37	1.38	1.31	1.37	1.42	0.140	0.012
Hatchability	100.0	94.64	97.50	97.50	97.50	0.662	1.051

SEM: Standard error of the mean. EW:Egg weight, HW: Hatching weight.

The results of the study show that the use of different carbohydrates in *in ovo* feding even though it does not have the same effect on the whole digestive system or its different parts, chicks with improved digestive system can be obtained with the carbohydrates used. In the present study, *in ovo* carbohydrates feeding did not decrease hatchability unlike Ebrahimnezhad et al. (glucose 2011), Zhai et al. (glucose, fructose, maltose, saccharose and dextrin, 2011a) and Salmanzadeh et al. (glucose and magnesium, 2012). This may be due to the difference in the amount of carbohydrate injected into the egg, *in ovo* injection time, and injection site in the present study (Kadam et al., 2013; Kop-Bozbay et al., 2016). Thus, in the present study, 0.25 mg carbohydrate was injected per egg, whereas in the other three studies 0.1-0.125 mg, 0.3 mg and 0.075-0.125 mg were injected from each carbohydrate, respectively. In addition, Ebrahimnezhad et al. (2011) and Salmanzadeh (2012) injected into the albumen on the 7th day of incubation, Zhai et al. (2011a) injected into the amnion on 18.5 days. Therefore, the results of the present study show that the dose used is suitable for the hatchability and that it is not allergic.

Table (2): Relative yolk sac and digestive system segments of chicks obtained from eggs without injection (NC), *in ovo* saline (PC), glucose (G), sucrose (S) and corn starch (CS) injected

	NC	PC	G	S	CS	P	SEM
Relative weight, %							
Yolk	15.70^{b}	13.09^{b}	18.70^{a}	13.48^{b}	15.11^{b}	<0.001	0.469
Gizzard	3.527^{ab}	3.592^{ab}	3.230^{b}	4.063^{a}	3.879^{a}	0.029	0.090
Proventriculus	0.591^{ab}	0.548^{b}	0.476^{b}	0.748^{a}	0.787^{a}	0.007	0.033
Duodenum	0.275^{b}	0.488^{ab}	0.308^{ab}	0.474^{ab}	0.546^{a}	0.041	0.036
Jejenum	0.424^{b}	0.405^{b}	0.330^{b}	0.597^{ab}	0.731^{a}	0.020	0.043
İleum	0.260	0.329	0.198	0.364	0.440	0.238	0.035
GIT	3.039^{b}	3.370^{ab}	3.066^{b}	3.818^{a}	3.325^{ab}	0.019	0.084
Relative lenght, %							
Duedenum	14.27	13.33	14.94	14.57	13.80	0.741	0.393
Jejenum	23.67	23.31	26.38	26.19	27.23	0.490	0.835
İleum	16.22^{b}	14.13^{b}	16.85^{b}	21.75^{a}	22.08^{a}	0.002	0.818
GIT	69.97^{c}	68.25^{c}	72.35^{bc}	82.67^{a}	81.03^{ab}	0.011	1.694

SEM: Standard error of the mean.a, b, c: Means within a rows not sharing common superscripts differ significantly ($p < 0.05$).

Table (3): Relative liver weights and liver color values (CIE Lab) of chicks obtained from eggs without injection (NC), *in ovo* saline (PC), glucose (G), sucrose (S) and corn starch (CS) injected

	NC	PC	G	S	CS	P	SEM
Weight, g	1.904^{b}	2.018^{ab}	1.775^{b}	2.300^{a}	2.348^{a}	0.002	0.057
CIE Lab							
L*	56.34	53.95	52.28	53.24	52.66	0.196	0.592
a*	22.10	25.20	23.78	24.44	24.17	0.596	0.603
b*	50.52	50.55	48.32	46.10	45.16	0.573	1.273

SEM: Standard error of the mean. a, b: Means within a rows not sharing common superscripts differ significantly ($p < 0.05$).

Energy resources for poultry embryos are relatively limited. Therefore, embryos use energy reserves in the liver and glycolytic muscles during hatching (Christensen et al., 2001), because they can metabolize glycerol and amino acid substrates as energy sources (Klasing, 1998). Inadequate and excessive consumption of glycogen reserve during hatching causes inhibition of growth leading to increased embryonic deaths (Donaldson and Christensen, 1994; Kop-Bozbay and Ocak, 2019). Therefore, in the present study, it can be said that the energies required by the embryo before the hatching can be provided exogenously with carbohydrates used in *in ovo* feeding.

There was a linear relationship between egg weight and hatchability (Göger et al., 2018) and it was determined that *in ovo* glucose injection increased hatching weight (Bhanja et al., 2008; Ebrahimnezhad et al., 2011; Kornasio et al., 2011; Zhai et al., 2011a; Salmanzadeh, 2012). In line with these reports, in the current study, *in ovo* G feeding increased hatching weight. Morever, the weight of the chicks obtained in the present

study (49.9 g) was higher than that of the chicks (average 40 g) in other studies (Ebrahimnezhad et al., 2011; Zhai et al., 2011a; Salmanzadeh, 2012). Egg mass and internal quality directly affect the ability of the embryo to access nutrients and provide resources to support chick self-sufficiency (Moran, 2007; Uni et al., 2003a, b). In the present study, our results on the ratio of egg weight to hatching weight show that the eggs used are sufficient in terms of related characteristics.

Liver glycogen and serum glucose concentrations are correctly correlated on the day of hatching (Tasharofi et al., 2018). Embryos have the ability to regulate their development according to the available nutrients in the egg (Uni et al., 2003a, b). Thus, the relative residual yolk weights of chicks obtained from eggs injected *in ovo* glucose were found approximately three units more than the other groups. As reported by Kop-Bozbay and Ocak (2019), this situation shows that embryo uses glucose as a ready-made energy source during the hatching process. In other words, glucose reduced the use of nutrients in the residual yolk sac (Zhai et al., 2011b). Sucrose is composed of glucose + fructose, while starch consists only of glucose units. This may explain why embryos use firstly glucose as a ready-made energy source.

Reaching maximum growth potential of embryos depends on the physical and physiological development of the digestive systems in the perinatal period (Tako et al., 2004; Cheled - Shoval et al., 2011; Jia et al., 2011). Studies on the perinatal development of the digestive system in poultry embryos (Geyra et al., 2001; Iji et al., 2001; Uni et al., 2003) have shown that nutrient uptake and digestion of embryos depend on the consumption of exogenous nutrients. Indeed, the functional development of the digestive system at hatch of the chick obtained by *in ovo* feeding was similar to that of a 2-day-old chick that immediately access feed and water after hatch (Uni et al., 2003). This may be an explanation for the increase in total digestive system weight and length of chicks obtained by *in ovo* S and CS feeding. Such a process may be the result of early stimulation of the digestive system (Kornasio et al., 2011).Positive effects on other parts of the digestive system (proventriculus, stony, etc.) originating from the *in ovo* S and CS injection support this thesis. The digestive system develops throughout the incubation as a whole, but the functional capabilities of the small intestine, extensive morphological, cellular and molecular changes in this organ, begin to develop only in the last quarter of the incubation (Uni et al., 2003b; Dibner and Richards, 2004; Kop-Bozbay and Ocak, 2019).

Sucrose and starch are more effective in the development of the digestive system and sections than *in ovo* glucose feeding, this may be related to the fact that the digestive system has worked more for the digestion of carbohydrates (Tako et al., 2004; Cheled - Shoval et al., 2011; Jia et al., 2011). Sklan (2001) reported that *in ovo* feeding increases villus surface area and height, which improves digestion and assimilation of nutrients. The higher efficiency of the *in ovo* sucrose and starch feeding on the physical development of the digestive system compared to the *in ovo* glucose feding may be due to the release of glucose from sucrose and starch and / or the properties of the carbohydrate-forming units such as absorption, transport and storage (Hocquette and Abe, 2000).This may explain why the carbohydrates used do not have the same stability. It is determined that there is a positive relationship between growth performance with a well-developed digestive system and hatching weight in poultry (Sklan, 2001). Therefore, due to the high hatching weight caused by *in ovo* G feeding and the well-developed digestive system caused by *in ovo* S and CS feeding, it is expected that the chicks obtained from these groups will have higher growth performance. However, this situation was not evaluated in the present study.

The embryonic liver grows intensely in the perinatal period by the embryo to meet the increased metabolic requirements for energy and nutrients (Tasharofi et al., 2018). Indeed, blood glucose is increasingly increasing to support the accumulation of glycogen in the liver and muscle (Kornasio et al., 2011). Therefore, the accumulation of glycogen in the embryonic liver was found to be associated with changes in liver cells due to the metabolism of carbohydrates taken from the embryo (glycogenesis and / or exogenous sources) (Moran, 2007). The lower liver weight in the glucose group may be attributed to the fact that the embryo provides the energy it needs from the liver stores because of the delayed absorption of glucose due to the digestion of sucrose and starch (Bhattacharyya et al., 2007). Thus, there is a change in embryo weight and growth of metabolically active organs due to the decrease in nutrient absorption and mobilization in the amount of yolk sac (Moran, 2007; Kornasio et al., 2011). In addition, the negative relationship between liver weight and residual yolk sac weight (Bhattacharyya et al., 2007) may also be an explanation for the results of this study. Indeed, the present study confirms this suggestion that the residual yolk sac weight was obtained in the Ggroup.

The results of the present study on metabolically active organs and tissues and liver color values supported the idea that the amount of residual yolk sac, changes in liver color and the ability of the gastrointestinal tract to digest and absorb exogenous nutrients (Moran, 2007). The rapid growth of the embryonic liver confirms that their function is crucial for normal embryo development. Therefore, higher liver weight at hatch may be an indicator of better energy metabolism in CS groups. Hence, starch injection in eggs may be a usefull way for the chicken embryo to use energy more efficiently (Uni et al., 2005).

IV. Conclusion

The results of this study show that even though the carbohydrates do not have the same effect on the whole digestive system or different parts of the *in ovo* feeding with different carbohydrates, chicks with higher digestive system and higher hatching weight can be obtained with the used carbohydrates. Thus, *in ovo* carbohydrate feeding may have a long-term effect on the development of metabolically active organs, thereby improving hatching weight by increasing liver and muscle glycogen reserves.

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