Nutrition of the Developing Embryo and Hatchling[1]

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ABSTRACT Nutrient needs central to satisfactory egg incubation well-being undergo several major changes from fertilization until the reliance of the chick on feed. Glucose is central, with the initiation of incubation until the chorioallantois accesses O_2 to use for fatty acid oxidation. Nutrient recovery from albumen and yolk is largely commensurate with body assembly through to completion of the embryo by 14 d. Remaining albumen mixes with the amniotic fluid and is orally consumed until initiation of emergence. A portion of the albumen is absorbed by the small intestine to expand body glycogen reserves. The residual not absorbed contains digestive enzyme contributions and enters the yolk sac through its stalk at the jejunum and ileum. Interaction of the albumen-amnion digestive enzyme mixture with yolk sac contents leads to diverse alterations that influence subsequent use of lipids. Rapid removal of very low-density lipoprotein ensues, until pipping with triglycerides, expanding body fat depots while cholesterol deposits in the liver. A concurrent translocation of Ca from shell mineralizes the skeletal system while also crossing yolk sac villi for deposition on phosvitin-based granules accruing in its lumen. Loss of chorioallantois with pipping and the start of pulmonary respiration predispose a dependence on glycolysis to support emergence. Small intestinal villi progressively reorient their enterocytes from macromolecule transfer to competence at digestion and absorption after hatching. Mobilization of body fat complements contributions from the yolk sac to provide fatty acids for generating energy, heat, and water while also combining with hepatic cholesterol for membrane expansion and continued development. Calcified granules evacuate the yolk sac to further skeletal mineralization in the absence of shell contributions. Egg mass, its interior quality, and turning during early incubation directly influence the ability of the embryo to access nutrients and provide resources to support emergence and the transition of the chick to self-sufficiency.

Key words: albumen, embryo nutrition, incubation, yolk sac

2007 Poultry Science 86:1043–1049

INTRODUCTION

Feeding essential nutrients to the hen must be followed with their placement in the egg (Wilson, 1997). Then, a synchronized progression of events ensues to ultimately realize a viable chick. Access to O_2, diversified energy sourcing, accommodating metabolic patterns, and emphasis at mineralization are particularly important and may be dramatically altered with each event. Their importance is emphasized by an accentuation of deaths at each transition to emphasize the magnitude of changes. Essentially, the germ is established during the first third of incubation, and completion of the embryo form follows through the second third. Preparation for emergence and actual hatching become the objectives during the final third. Finally, establishing an independent chick depends on embryonic reserves in place to fuel its transition to feed. Considerable poetic license has been taken to assemble a multitude of isolated research observations into a holistic view of embryo and hatchling nutrition. Providing a multidisciplinary model rationalizes existing strategies known to improve hatchability and subsequent chick performance.

ESTABLISH GERM

Germ development is initiated with ovum fertilization in the infundibulum of the hen and continues with incubation of the subsequent egg. Nutrients initially supporting of the germ are recovered from adjacent yolk (Tezuka et al., 1974; Litke and Low, 1975; Lemanski and Aldoroty, 1977) and albumen (Deeming, 1989a) by an expanding vascular system (Ribatti, 1995). Although a germ vascular system is visually apparent with candling shortly after incubation, O_2 access is limited to simple diffusion aided by a primitive hemoglobin (Cirotto and Arangi, 1989; Baumann and Meuer, 1992). Energy expended at this time largely arises by glycolysis of accessible glucose, and a transient increase of lactic acid occurs until the chorioallantois becomes functional (Kucera et al., 1984). Glucose

Received November 13, 2006.

Accepted November 19, 2006.

[1]Presented as part of the Embryo Symposium: Managing the Embryo for Performance, July 19, 2006, at the Poultry Science Association Annual Meeting, Edmonton, Alberta, Canada.

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in albumen appears to be largely recovered from the outer thin and put in place during uterine plumping before shell formation. Simplistically, invagination during early development of the germ leads to the chorionic sac and allantoic cavity. In turn, their membranes eventually converge at the shell to favor distribution at the air-cell end of the egg (Sethi and Brookes, 1971; Fancsi and Feher, 1979). Vascular development adjacent to these membranes enables a rapid exchange of CO_2 and O_2 with shell air pores (Tazawa and Ono, 1974; Lomholt, 1976; Tullety and Board, 1976). Access to O_2 fully supports complete combustion of fatty acids, used as the primary source of energy and the basis for embryo development.

Albumen structure is a dominant factor to the successful transition of the germ from anaerobic to aerobic metabolism (Christensen, 2001). Essentially, the chalaza, along with thick albumen, restricts ovum movement to rotation within the inner thin of the egg (Tullet and Deeming, 1987; Deeming, 1989b). Extensive lipid content encourages the yolk to rise within this aqueous system such that chorion and amnion membranes favor juxtaposition at the inner eggshell membrane while improving survival by proximity to glucose in the outer thin. Although differential pressure treatment of turkey eggs before incubation can be used to increase egg glucose content, Moran and Reinhart (1981) did not observe a relief in dead-germ loss as much as increased poult weight. Frequent egg turning by the hen throughout early incubation fosters a uniformly distributed chorioallantois through the top half of the egg while continuing glucose access from the outer thin. Success of this transition is substantially dependent on albumen integrity. Long holding, poor holding, or both conditions before incubation not only impair formation of a fully functional chorioallantois but adversely define the albumen sac that concurrently forms at the small end and recovery of its nutrients (Hall and Van Wagenen, 1936; Christensen et al., 2001; Tona et al., 2005).

EMBRYO COMPLETION

The vascular system is fully developed shortly after the chorioallantois is complete to assure O_2-CO_2 exchange (Levinsohn et al., 1984). In turn, fatty acid use represents the most favorable energy source for completion of the embryo in its final form (Sato et al., 2006). A continuous low respiratory quotient infers that carbohydrate is being conserved to the extent that ketone body accumulation does not occur. Not only is adenosine triphosphatase provided in major amounts as the driver of synthetic processes, but major amounts of by-product heat and water also evolve in support of incubation (Whittow and Tarazawa, 1991). An increased proportion of the essential fatty acids appearing in egg contents with the progression of incubation indicates their devotion to membrane formation in embryonic assembly while saturates with their greater caloric value are consumed (Donaldson, 1964; Maldjian et al., 1995; Murray et al., 1999; Speake et al., 2003; Speake and Deans, 2004). Nutritional deficiencies imposed on the breeder during egg formation may have repercussions at any time, but embryonic deaths during this interval usually relate to marginal breeder feed inadequacies. Inadequacies of pantothenic acid and riboflavin may occur in practice, and accentuated deaths at 12 d of incubation together with clubbed down are observed. Although the embryo is structurally complete by 14 d of incubation, providing the wherewithal to support the transition to an independent chick in the last 7 d is very demanding as well.

PREPARATIONS FOR EMERGENCE

Embryo expansion within the amniotic sac together with its physical movements initiate preparations for emergence. Albumen previously compartmentalized at the small end of the egg has its seroamniotic connection rupture (Feher, 1984). Free albumen can now enter the amniotic sac and form a composite of both contents. Pressure differential created by a continuing embryonic growth enhances an oral consumption of this mixture that passes through the gastrointestinal system (Oegema and Jourdian, 1974; Sugimoto et al., 1999). Partial absorption of proteins from the albumen-amniotic mixture occurs by enterocytes capable of macromolecular absorption during transit through the duodenum and jejunum. Antitrypsin factors strongly inhibit digestion, even though a full complement of pancreatic enzymes is present (Holdsworth and Wilson, 1967; Marchaim and Kulka, 1967; Yoshizaki et al., 2002). Albumen, together with the pancreatic enzyme composite, enters the yolk sac through its stalk with the aid of antiperistaltic activity of the colon (Sugimoto et al., 1989; Bryk and Gheri, 1990). Recovery of macromolecules during passage through the duodenum and jejunum is parallel to that of colostrums with postparturient mammals. Chick embryonic villi also have unique enterocytes that are capable of fluid and macromolecular transfer to greatly expand the vascular system with the appearance of albumen proteins. Such consumption and absorption is continuous until the albumen-amniotic fluid disappears and internal pipping begins. Ovomucoid is a prominent albumen protein that appears in blood and continues to be detectable after hatching. This protein is one of several in albumen that has extensive amounts of carbohydrate. Gluconeogenesis from albumen proteins focuses on using the inherent carbohydrate to form glycogen while conserving amino acids per se for protein synthesis (Scott et al., 1981; Muramatsu et al., 1990). As a result, blood glucose progressively increases to support escalating liver and muscle glycogen deposition.

Residual albumen-allantoic fluid entering the yolk continues to express digestive enzymes released en route through the gastric and small intestinal systems (Bainter and Feher, 1974; Sugimoto et al., 1978; Sugimoto and Yamada, 1986a,b; Ikeno and Ikeno, 1991). Ovomucoid is known to strongly inhibit trypsin; however, subsequent encounters of the enzyme composite with lipoproteins in the yolk sac appear to foster major molecular changes. The yolk sac has villi that expand surface area in parallel to the intestine (Holdsworth and Wilson, 1967). Similarly,

core vessels have an extensive vascular system to facilitate exchange dynamics; however, yolk sac cells on villi use receptor-mediated endocytosis to absorb intact very low density lipoproteins (**VLDL**) rather than transport digestion products (Lambson, 1970; Noble et al., 1988). These cells also have a diverse array of enzymes capable of altering the absorbed lipid before release into circulation (Kusuhara and Ishida, 1974; Powell et al., 2004). Very low-density lipoprotein uptake by the yolk sac becomes greatly accentuated beyond simple needs once the albumen-allantoic digestive enzyme composite is encountered (Speake et al., 1992). Specialized depots that develop in many subdermal locations of the embryo accept this additional transfer of triglycerides, whereas associated cholesterol preferentially locates in the liver to exaggerate its size and create a distinctive light yellow appearance (McGreal, 1956; Langslow and Lewis, 1972; Speake et al., 1998; Peebles et al., 1999).

Phosvitin and lipovitellin are yolk high-density lipoproteins formed from vitellogenin during transfer through the ovum wall. Associated changes lead to molecular rearrangement into granules that aggregate into spheres apart from the aqueous dispersion of VLDL. Concurrent with entry of albumen-allantoic fluid enzymes in the yolk sac, sphere appearance changes from an accrual of granules to a lamination of layers from surface to core (Cheville and Coignoul, 1984). Phosvitin has a large amount of phosphate esterified to serine (Shainkin and Perlmann, 1971a,b; Perlmann, 1973), and lamination appears to enable to direct exposure of large amounts of these phosphates at the surface. Calcium can be transferred through yolk sac villi from its vascular system to adhere to the surface of the sphere. Calcified granules are not absorbed but accrue within the confines of the yolk sac until pipping.

Calcium in circulation increases dramatically, concurrent with the transfer of VLDL from the yolk sac to embryo depots and sphere calcification. Dissolution of mammary knobs adjacent to the chorioallantois-shell membrane interface represents the dominant source of blood Ca (Bond et al., 1988; Dieckert et al., 1989; Abdel-Salam et al., 2006). Exchange of CO_2 in conjunction with carbonic anhydrase creates acid conditions for the dissolution of the shell and transfer of Ca to circulation (Narbaitz, 1974; Tuan, 1984, 1987; Tuan and Ono, 1986). Calcium so recovered not only calcifies yolk sac spheres but has the intention of concurrently mineralizing a developing skeleton that was previously cartilage (Roufosse, 1979).

IMPLEMENTING RESOURCES FOR EMERGENCE

Internal pipping initiates emergence by piercing the chorioallantois and inner shell membrane at the air cell periphery. Loss of ready O_2 access necessitates a concurrent transition to pulmonary respiration, because the outer shell membrane and shell are progressively circumscribed (Vince, 1976; Menna and Mortola, 2002; Villamor et al., 2002). Essentially, shell piercing by the beak is driven by the hatching muscle as facilitated by the egg "tooth" of the beak and body rotation (Bakhuis, 1974; Feher, 1988). Hatching muscle fibers are exclusively anaerobic, very well provided with glycogen, and have a special nervous system for coordination (Fazekas et al., 1985; Gross, 1985; John et al., 1987). Maximum vascularization at the air cell-shell interface also lead to shell areas weakened during the 14 to 19 d of accentuated Ca recovery commensurate with areas fractured (Waters, 1935).

Cessation of shell Ca access with chorioallantois destruction initiates the recovery of Ca phosphate high-density lipoprotein spheres from the yolk sac (Cheville and Coignoul, 1984). Transition from sourcing Ca at the shell to both Ca and P in the yolk sac provides a continued mineralization and skeletal development through to feed dependence. Continual skeletal reinforcement is paramount to supporting body emergence from the shell and subsequent locomotion (Pageze et al., 1996). Sphere Ca-P access largely diminishes within 3 to 4 d after hatch with yolk sac depletion, and its amount likely influences early feed macromineral requirements. Hen dietary P seems to influence vitellogenin formation and sphere levels during ovum formation. Small egg mass may be of substantial influence in this respect (El Boushy, 1979a,b; Triyuwanta et al., 1992).

The physical demands of emergence are extensive when O_2 availability is marginal (Tazawa et al., 1983). Muscles most active at this time exclusively use glycolysis from glucose provided from glycogen reserves (Freeman, 1969). The transient increase of lactic acid occurring at this time disappears once pulmonary functioning provides adequate O_2 for fatty acid catabolism to resume as a source of energy (Garcia et al., 1986; Hoiby et al., 1987). Although respiratory quotient indicates that fatty acid oxidation dominates energy production once emergence is complete, access to glucose remains important to fully combust fatty acids without ketone body accumulation (Best, 1966; Beis, 1985; Ohtsu et al., 2003). By-product heat from fatty acid combustion is substantial and assists in transition of the neonate from being a poikilotherm to homeothermy, whereas the associated production of water production minimizes dehydration until external sources become available.

Although yolk sac lipoproteins continue to be an important nutrient source at this time (Romanoff, 1944; Noble and Ogunyemi, 1989; Castillo et al., 1992; Murakami et al., 1992; Nir and Levanon, 1993; Ding and Lilburn, 1996; Puvadolpirod et al., 1997), fatty acid recovery from preformed body depots appears to dominate (Langslow, 1972) given the minimal repercussion of deutectomy (Harvey et al., 1955; Baranyiova, 1972; Baranyiova and Standara, 1980). Extensive hepatic cholesterol in place at hatch rapidly dissipates along with the depots (Svanberg, 1971; Baranyiova and Holman, 1972; Tarugi et al., 1994). Immediate access to cholesterol appears to relieve its synthesis. Cholesterol, together with depot essential fatty acids, enables continued membrane formation and growth. A high concurrent demand for glucose appears to reside with its need to support growth of glycolytic

muscle (Baranyiova and Holub, 1977; Latour et al., 1995, 1996; Uni et al., 2005). End-product pyruvic acid (Ala) recycles for regeneration in the liver using fatty acid energy.

TRANSITION TO FEED

The gastrointestinal system must assume responsibility for nutrition of the chick as reserves originating from the egg deplete (Jin et al., 1998; Vieira and Moran, 1999). Enterocytes in place with the villi of the embryo are intended for macromolecular transfer; however, those arising from the crypts shortly replace embryonic ones to provide competence at digestion-absorption (Uni et al., 1998, 2003a,b). A mosaic of both type of enterocytes exists at emergence, and another several days must ensue before effective feed utilization is possible (Raheja et al., 1977; Sulistiyanto et al., 1999; Batal and Parsons, 2002; Sklan, 2003). Essentially, the entire surface makes this transition within 2 wk, and all remnants of the embryonic population are extruded. Growth of the mucosa through this period almost exclusively represents villi, and the increased appearance of enzymes that finalize digestion correspond to surface maturation (Baranyiova and Holman, 1976; Noy and Sklan, 1995; Uni et al., 1995; Chotinsky et al., 2001; Iji et al., 2001). Loss of yolk sac, disappearance of subdermal depots, and resumption of liver color must be in phase with the ability of the gastrointestinal system to recover nutrients from feed. Most starveouts fail to make the transition and commonly maximize around 3 to 4 d posthatch. The multitude of strategies that provide early nutrition to either an embryo or postemergent chick provide an advantage to this transition, thereby improving performance (Baranyiova, 1987; Noy and Pinchasov, 1993; Ohta et al., 1999; Tako et al., 2004; Foye et al., 2006a,b).

OVERVIEW

A holistic view of embryo and hatchling nutrition indicates a central role for carbohydrate, fatty acids, and Ca and P. Transient access to O_2 until establishment of the germ and again during emergence of the chick favors anaerobic catabolism of glucose for energy. Internal egg quality and turning during early incubation are mutually necessary for favorable formation of the chorioallantois and compartmentalizing remaining albumen. Effective retrieval of O_2 enables fatty acid oxidation to dominate energy production during the interim until emergence and immediately after its completion. Oral consumption of albumen once the embryo is formed and partial absorption of the protein provides for gluconeogenesis and storage of glycogen to fuel emergence. Remaining albumen together with digestive enzymes enter the yolk sac to facilitate other changes. Yolk VLDL are rapidly absorbed to expand body depots while cholesterol accrues in the liver. Transfer of Ca from the eggshell periphery weakens these areas for pipping. Part of this Ca mineralizes the skeleton, whereas a significant amount is also transferred onto phosvitin-based platelets in the yolk sac. Calcium phosphate platelets provide continued skeletal mineralization after loss of the chorioallantois. Upon hatching, enterocytes competent at food assimilation subsequently cover the surface of the intestine. Body depots and hepatic cholesterol in the posthatch chick are recalled as the yolk sac depletes and provide continued body development during the transition to self-sufficiency.

REFERENCES

Abdel-Salam, Z. A., A. M. Abdou, and M. A. Harith. 2006. Elemental and ultrastructural analysis of the eggshell: Ca, Mg, and Na distribution during embryonic development via LIBS and SEM techniques. Int. J. Poult. Sci. 5:35–42.

Bainter, K., and G. Feher. 1974. Fate of egg white trypsin inhibitor and start of proteolysis in developing chick embryo and newly hatched chick. Dev. Biol. 36:272–278.

Bakhuis, W. L. 1974. Observations on hatching movements in the chick (*Gallus domesticus*). J. Comp. Physiol. Psychol. 87:997–1003.

Baranyiova, E. 1972. Influence of deutectomy, food intake and fasting on liver glycogen content in chickens after hatching. Acta Vet. Brno 41:149–159.

Baranyiova, E. 1987. Effect of intraperitoneal administration of amino acids on the food intake of chickens in the first month after hatching. Acta Vet. Brno 56:417–426.

Baranyiova, E., and J. Holman. 1972. Lipid droplets in hepatocytes of chicken embryos and chickens after hatching. Acta Vet. Brno 41:367–372.

Baranyiova, E., and J. Holman. 1976. Morphological changes in the intestinal wall in fed and fasted chickens in the first week after hatching. Acta Vet. Brno 45:151–158.

Baranyiova, E., and A. Holub. 1977. Influence of glucose load on food intake of chickens during the early posthatching period. Acta Vet. Brno 46:237–244.

Baranyiova, E., and S. Standara. 1980. Effect of deutectomy, fasting and environmental temperature on free fatty acid concentration in blood plasma of chickens in the first week after hatching. Acta Vet. Brno. 49:187–192.

Batal, A. B., and C. M. Parsons. 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. 81:400–407.

Baumann, R., and H. J. Meuer. 1992. Blood oxygen transport in the early avian embryo. Physiol. Rev. 72:941–965.

Beis, A. 1985. Activities of the enzymes of ketone body metabolism in the developing chick. Comp. Biochem. Physiol. B 81:671–675.

Best, E. E. 1966. The changes of some blood constituents during the initial post-hatching period in chickens. II. Blood total ketone bodies and the reduced glutathionine ketone body relationship. Br. Poult. Sci. 7:23–28.

Bond, G. M., R. G. Board, and D. V. Scott. 1988. A comparative study of changes in the fine structure of avian eggshells during incubation. Zoolog. J. Linn. Soc. 92:105–113.

Bryk, S. G., and Z. G. Gheri. 1990. On the development of the chick embryo colon: A computerized morphometric study. Z. Mikrosk. Anat. Forsch. 104:97–118.

Castillo, M., M. F. Zafra, F. Rodríguez-Vico, J. M. Lopez, and E. Garcia-Peregrin. 1992. Changes in the chick lipoprotein profile during postnatal development. Biochem. Arch. 8:183–190.

Cheville, N., and F. Coignoul. 1984. Calcium in the turkey yolk sac during late development and postembryonic evolution. Am. J. Vet. Res. 45:2458–2466.

Chotinsky, D., E. Toncheva, and Y. Profirov. 2001. Development of disaccharidase activity in the small intestine of broiler chickens. Br. Poult. Sci. 42:389–392.

Christensen, V. L. 2001. Factors associated with early embryonic mortality. World's Poult. Sci. J. 57:359–372.
Christensen, V. L., M. J. Wineland, G. M. Fasenko, and W. E. Donaldson. 2001. Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. Poult. Sci. 80:1729–1735.
Cirotto, C., and I. Arangi. 1989. How do avian embryos breath? Oxygen transport in the blood of early chick embryos. Comp. Biochem. Physiol. A 94:607–613.
Deeming, D. C. 1989a. Importance of sub-embryonic fluid and albumen in the embryo's response to turning of the egg during incubation. Br. Poult. Sci. 30:591–606.
Deeming, D. C. 1989b. Characteristics of unturned eggs: Critical period, retarded embryonic growth and poor albumen utilization. Br. Poult. Sci. 30:239–249.
Dieckert, J. W., M. C. Dieckert, and C. R. Creger. 1989. Calcium reserve assembly: A basic structural unit of the calcium reserve system on the hen egg shell. Poult. Sci. 68:1569–1584.
Ding, S. T., and M. S. Lilburn. 1996. Characterization of changes in yolk sac and liver lipids during embryonic and early posthatch development of turkey poults. Poult. Sci. 75:478–483.
Donaldson, W. E. 1964. Fatty acid composition of chick embryo and egg yolk at various stages of incubation. Poult. Sci. 43:784–785.
El Boushy, A. R. 1979a. Available phosphorus in poultry. 1. Effect of phosphorus levels on the performance of laying hens and their egg quality, hatchability, bone analysis and strength in relation to calcium and phosphorus in the blood. Neth. J. Agric. Res. 27:176–183.
El Boushy, A. R. 1979b. Available phosphorus in poultry. 2. Effect of phosphorus in diet on performance of chicks, bone composition and strength, and calcium and inorganic phosphorus in blood plasma. Neth. J. Agric. Res. 27:184–189.
Fancsi, T., and G. Feher. 1979. Ultrastructural studies of chicken embryo chorioallantoic membrane during incubation. Anat. Histol. Embryol. 8:151–159.
Fazekas, S., G. Feher, L. Kondics, I. Ovary, and V. Szekessy-Hermann. 1985. Purification and properties of myosin from the "hatching muscle" (*M. complexus*) of geese. Acta Physiol. Hung. 66:5–25.
Feher, G. 1984. Development of the albumen sac and changes of its structure in the goose during hatching. Magy. Allatorvosok Lapja 39:105–112.
Feher, G. 1988. The process of hatching in goose and duck. Anat. Histol. Embryol. 17:107–120.
Foye, O. T., Z. Uni, and P. R. Ferket. 2006a. Effect of in ovo feeding egg white protein, β-hydroxy-β-methylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys. Poult. Sci. 85:1185–1192.
Foye, O. T., Z. Uni, J. P. McMurtry, and P. R. Ferket. 2006b. The effects of amniotic nutrient administration, "in ovo feeding" of arginine and/or β-hydroxy-β-methyl butyrate (HMB) on insulin-like growth factors, energy metabolism and growth in turkey poults. Int. J. Poult. Sci. 5:309–317.
Freeman, B. M. 1969. The mobilization of hepatic glycogen in *Gallus domesticus* at the end of incubation. Comp. Biochem. Physiol. 28:1169–1176.
Garcia, F. J., A. Pons, M. Alemany, and A. Palout. 1986. Tissue glycogen and lactate handling by the developing domestic fowl. Comp. Biochem. Physiol. A 85:155–159.
Gross, G. H. 1985. Innervation of the complexus ("hatching") muscle of the chick. J. Comp. Neurol. 232:180–189.
Hall, G. O., and A. Van Wagenen. 1936. The association of certain measures of interior egg quality with hatchability. Poult. Sci. 15:501–506.
Harvey, J. D., D. B. Parrish, and P. E. Sanford. 1955. Improvements in the technique of deutectomy of newly hatched chicks, and the effects of the operation on their subsequent development. Poult. Sci. 34:3–8.
Hoiby, M., A. Aulie, and P. O. Bjonnes. 1987. Anaerobic metabolism in fowl embryos during normal incubation. Comp. Biochem. Physiol. A 86:91–94.
Holdsworth, C. D., and T. H. Wilson. 1967. Development of active sugar and amino acid transport in the yolk sac and intestine of the chicken. Am. J. Physiol. 212:233–240.
Iji, P. A., A. Saki, and D. R. Tivey. 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. Br. Poult. Sci. 42:514–522.
Ikeno, T., and K. Ikeno. 1991. Amylase activity increases in the yolk of fertilized eggs during incubation in chickens. Poult. Sci. 70:2176–2179.
Jin, S.-H., A. Corless, and J. L. Sell. 1998. Digestive system development in post-hatch poultry. World's Poult. Sci. J. 54:335–345.
John, T. M., J. C. George, and E. T. Moran Jr. 1987. Pre- and post-hatch ultrastructural and metabolic changes in the hatching muscle of turkey embryos from antibiotic and glucose treated eggs. Cytobios 49:197–210.
Kucera, P., E. Raddatz, and A. Baroffio. 1984. Oxygen and glucose uptake in the early chick embryo. Pages 299–309 in Respiration and Metabolism of Embryonic Vertebrates. R. S. Seymour, ed. W. Junk, Dordrecht, the Netherlands.
Kusuhara, S., and K. Ishida. 1974. Histochemical observations on the enzymes of chicken yolk sac membrane. Br. Poult. Sci. 15:391–394.
Lambson, R. O. 1970. An electron microscopic study of the endodermal cells of the yolk sac of the chick during incubation and after hatching. Am. J. Anat. 129:1–20.
Langslow, D. R. 1972. The development of lipolytic sensitivity in the isolated fat cells of *Gallus domesticus* during the foetal and neonatal period. Comp. Biochem. Physiol. B 43:689–701.
Langslow, D. R., and R. J. Lewis. 1972. The compositional development of adipose tissue in *Gallus domesticus*. Comp. Biochem. Physiol. B 43:681–688.
Latour, M. A., E. D. Peebles, C. R. Boyle, J. D. Brake, and T. F. Kellog. 1995. Changes in serum lipid, lipoprotein and corticosterone concentrations during neonatal chick development. Biol. Neonate 67:381–386.
Latour, M. A., E. D. Peebles, C. R. Broyle, S. M. Doyle, T. Pansky, and J. D. Brake. 1996. Effects of breeder hen age and dietary fat on embryonic and neonatal broiler serum lipids and glucose. Poult. Sci. 75:695–701.
Lemanski, L. F., and R. Aldoroty. 1977. Role of acid phosphatase in the breakdown of yolk platelets in developing amphibian embryos. J. Morphol. 153:419–426.
Levinsohn, E. M., D. S. Packard Jr., E. M. West, and D. R. Hootnick. 1984. Arterial anatomy of chicken embryo and hatchling. Am. J. Anat. 169:377–405.
Litke, L. L., and F. N. Low. 1975. Scanning electron microscopy of yolk absorption in early chick embryos. Am. J. Anat. 142:527–531.
Lomholt, J. P. 1976. The development of oxygen permeability of the avian egg shell and its membranes during incubation. J. Exp. Zool. 198:177–184.
Maldjian, A., K. Farkas, R. C. Noble, M. Cocchi, and B. M. Speake. 1995. The transfer of docosahexaenoic acid from yolk to the tissues of the chick embryo. Biochim. Biophys. Acta 258:81–89.
Marchaim, U., and R. G. Kulka. 1967. The non-parallel increase of amylase, chymotrypsinogen and procarboxypeptidase in the developing chick pancreas. Biochim. Biophys. Acta 146:553–559.
McGreal, R. D. 1956. Observations on the composition of the subcutaneous fat of the White Leghorn chick embryo. Poult. Sci. 35:1066–1069.
Menna, T. M., and J. P. Mortola. 2002. Metabolic control of pulmonary ventilation in the developing chick embryo. Respir. Physiolol. Neurobiol. 130:43–55.
Moran, E. T., Jr., and B. S. Reinhart. 1981. Providing glucose to turkey hatching eggs via pressure differential antibiotic dipping: Effects on hatchability, poult quality, and subsequent performance. Poult. Sci. 60:1544–1548.

Murakami, H., Y. Akiba, and M. Horiguchi. 1992. Growth and utilization of nutrients in newly-hatched chick with or without removal of residual yolk. Growth Dev. Aging 56:75–84.
Muramatsu, T., K. Hiramoto, N. Koshi, J. Okamura, S. Miyoshi, and T. Mitsumoto. 1990. Importance of albumen content in whole-body protein synthesis of the chicken embryo during incubation. Br. Poult. Sci. 31:101–106.
Murray, A. M., R. Denis, and B. K. Speake. 1999. Acyltransferase activities in the yolk sac membrane of the chick embryo. Lipids 34:929–935.
Narbaitz, R. 1974. On the mechanism of the shell mineral solubilization by the chick embryo. Calcif. Tissue Res. 16:339–342.
Nir, I., and M. Levanon. 1993. Effect of posthatch holding time on performance and on residual yolk and liver composition. Poult. Sci. 72:1994–1997.
Noble, R. C., and D. Ogunyemi. 1989. Lipid changes in the residual yolk and liver of the chick immediately after hatching. Biol. Neonate 56:228–236.
Noble, R. C., S. G. Tullett, and N. Yafei. 1988. Understanding the chick embryo V. A close view of the uptake of yolk fat. Poult. Misset. 4:32–33.
Noy, Y., and Y. Pinchasov. 1993. Effect of a single intubation of nutrients on subsequent early performance of broiler chicks and turkey poults. Poult. Sci. 72:1861–1866.
Noy, Y., and D. Sklan. 1995. Digestion and absorption in the chick. Poult. Sci. 74:366–373.
Oegema, T. R., and G. W. Jourdian. 1974. Metabolism of ovomucoid by the developing chick embryo. J. Exp. Zool. 189:147–161.
Ohta, Y., N. Tsushima, K. Koide, M. T. Kidd, and T. Ishibashi. 1999. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poult. Sci. 78:1493–1498.
Ohtsu, H., K. Sato, H. Nishida, and Y. Akiba. 2003. High β-hydroxybutyrate concentration in liver and skeletal muscle of newly hatched chicks. Comp. Biochem. Physiol. A 134:625–629.
Pageze, L., M. Manesse, M. Navarro, A. Carretero, M. Arboix, J. Ruberte, and J. Sautet. 1996. The morphometric study of the 19-day chicken embryo skeleton (*Gallus domesticus*). Vet. Hum. Toxicol. 38:299–301.
Peebles, E. D., L. Li, S. Millar, T. Pansky, S. Whitmarsh, M. A. Latour, and P. D. Gerard. 1999. Embryo and yolk compositional relationships in broiler hatching eggs during incubation. Poult. Sci. 78:1435–1442.
Perlmann, G. E. 1973. Phosvitin, a phosphoglycoprotein. Isr. J. Chem. 11:2–3.
Powell, K. A., E. A. Deans, and B. K. Speake. 2004. Fatty acid esterification in the yolk sac membrane of the avian embryo. J. Comp. Physiol. B 174:163–168.
Puvadolpirod, S., J. R. Thompson, J. Green, M. A. Latour, and J. P. Thaxton. 1997. Influence of yolk on blood metabolites in perinatal and neonatal chicks. Growth Dev. Aging 61:39–45.
Raheja, K. L., J. Tepperman, and H. M. Tepperman. 1977. The effect of age on intestinal glucose transport in the chick (*Gallus domesticus*). Comp. Biochem. Physiol. A 58:245–248.
Ribatti, D. 1995. A morphometric study of the expansion of the chick area vasculosa in shell-less culture. J. Anat. 186:639–644.
Romanoff, A. L. 1944. Avian spare yolk and its assimilation. Auk 61:235–241.
Roufosse, A. H. 1979. Identification of brushite in newly deposited bone mineral from embryonic chicks. J. Ultrastruct. Res. 68:235–255.
Sato, M., T. Tachibana, and M. Furuse. 2006. Heat production and lipid metabolism in broiler and layer chickens during embryonic development. Comp. Biochem. Physiol. A 143:382–388.
Scott, T. R., W. A. Johnson, D. G. Satterlee, and R. P. Gildersleeve. 1981. Circulating levels of corticosterone in the serum of developing chick embryos and newly hatched chicks. Poult. Sci. 60:1314–1320.
Sethi, N., and M. Brookes. 1971. Ultrastructure of blood vessels in the chick allantois and chorioallantois. J. Anat. 109:1–15.
Shainkin, R., and G. E. Perlmann. 1971a. Phosvitin, a phosphoglycoprotein: Composition and partial structure of carbohydrate moiety. Arch. Biochem. Biophys. 145:693–700.
Shainkin, R., and G. E. Perlmann. 1971b. Phosvitin, a phosphoglycoprotein. I. Isolation and characterization of glycopeptide from phosvitin. J. Biol. Chem. 246:2278–2284.
Sklan, D. 2003. Fat and carbohydrate use in posthatch chicks. Poult. Sci. 82:117–122.
Speake, B. K., and E. A. Deans. 2004. Biosynthesis of oleic, arachidonic and docosahexaenoic acids from their C_{18} precursors in the yolk sac membrane of the avian embryo. Comp. Biochem. Physiol. B 138:407–414.
Speake, B. K., E. A. Deans, and K. A. Powell. 2003. Differential incorporation of docosahexaenoic and arahidonic acids by the yolk sac membrane of the avian embryo. Comp. Biochem. Physiol. B 136:357–367.
Speake, B. K., A. M. Murray, and R. C. Noble. 1998. Transport and transformations of yolk lipids during development of the avian embryo. Prog. Lipid Res. 37:1–32.
Speake, B. K., R. C. Noble, and R. McCartney. 1992. Lipoprotein lipase activity in tissues of the developing embryo. Biochem. Soc. Trans. 20:295S.
Sugimoto, Y., T. Okita, and K. Koga. 1978. Detection of protease inhibitors in the egg yolk of hen and alteration of their activities during embryonic development. Jpn. Agric. Chem. 52:457–462.
Sugimoto, Y., A. Saito, T. Kusakabe, K. Hori, and K. Koga. 1989. Flow of egg white albumin into the yolk sac during embryogenesis. Biochim. Biophys. Acta 992:400–403.
Sugimoto, Y., S. Sanuki, S. Ohsako, Y. Higashimoto, M. Kondo, J. Kurawaki, H. R. Ibrahim, T. Aoki, T. Ksusakabe, and K. Koga. 1999. Ovalbumin in developing chicken eggs migrates from egg white to embryonic organs while changing its conformation and thermal stability. J. Biol. Chem. 274:11030–11037.
Sugimoto, Y., and M. Yamada. 1986a. Purification and characterization of benzoyl-L-tyrosine ethyl ester hydrolase from the yolk sac membrane of chicken egg. Biochem. Cell Biol. 64:543–547.
Sugimoto, Y., and M. Yamada. 1986b. Changes in proteins from yolk and in the activity of benzoyl-L-tyrosine ethyl ester hydrolase from the yolk sac membrane during embryonic development of the chicken. Poult. Sci. 65:789–794.
Sulistiyanto, B., Y. Akiba, and K. Sato. 1999. Energy utilization of carbohydrate, fat and protein sources in newly hatched broiler chicks. Br. Poult. Sci. 40:653–659.
Svanberg, O. 1971. The distribution of the yolk cholesterol in the young chick. An autoradiographic study. Acta Physiol. Scand. 83:373–381.
Tako, P., P. R. Ferket, and Z. Uni. 2004. Effects of in ovo feeding of carbohydrates and β-hydroxy-β-methylbutyrate on the development of chicken intestine. Poult. Sci. 83:2023–2028.
Tarugi, P., S. Nicolini, L. Marchi, G. Ballarini, and S. Calandra. 1994. Apolipoprotein B-100 production and cholesteryl ester content in the liver of developing chick. J. Lipid Res. 35:2019–2031.
Tazawa, H., and T. Ono. 1974. Microscopic observation of the chorioallantoic capillary bed of chicken embryos. Respir. Physiol. 20:81–89.
Tazawa, H., A. H. J. Visschedijk, J. Wittmann, and J. Piiper. 1983. Gas exchange, blood gases and acid-base status in the chick before, during and after hatching. Respir. Physiol. 53:173–185.
Tezuka, Y., Y. Yokoe, and K. Yamagami. 1974. Glycogen phosphorylases in yolk sac and liver of developing chick. Annot. Zool. Jpn. 47:74–83.
Tona, K., V. Bruggeman, O. Onagbesan, F. Bamelis, M. Gbeassor, K. Mertens, and E. Decuypere. 2005. Day-old chick quality: Relationship of hatching egg quality, adequate incubation

practice and prediction of broiler performance. Avian Poult. Biol. Rev. 16:109–119.
Triyuwanta, C. Leterrier and Y. Nys. 1992. Dietary phosphorus and food allowance of dwarf breeders affect reproductive performance of hens and bone development of progeny. Br. Poult. Sci., 33:363-379.
Tuan, R. S. 1984. Carbonic anhydrase and calcium transport function of the chick embryonic chorioallantoic membrane. Ann. N. Y. Acad. Sci. 429:459–472.
Tuan, R. S. 1987. Mechanism and regulation of calcium transport by the chick embryonic chorioallantoic membrane. J. Exp. Zool. Suppl. 1:1–13.
Tuan, R. S., and T. Ono. 1986. Regulation of extraembryonic calcium mobilization by the developing chick embryo. J. Embryol. Exp. Morphol. 97:63–74.
Tullett, S. G., and R. G. Board. 1976. Oxygen flux across the integument of the avian egg during incubation. Br. Poult. Sci. 17:441–450.
Tullet, S. G., and D. C. Deeming. 1987. Failure to turn eggs during incubation: Effects on embryo weight, development of the chorioallantois and absorption of albumen. Br. Poult. Sci. 28:239–243.
Uni, Z., P. R. Ferket, E. Tako, and O. Kedar. 2005. In ovo feeding improves energy status of late-term chicken embryos. Poult. Sci. 84:764–770.
Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult. Sci. 74:1622–1629.
Uni, Z., R. Platin, and D. Sklan. 1998. Cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the villus. J. Comp. Physiol. B 168:241–247.
Uni, Z., A. Smirnov, and D. Sklan. 2003a. Pre- and post-hatch development of goblet cells in the broiler small intestine: Effect of delayed access to feed. Poult. Sci. 82:320–327.
Uni, Z., E. Tako, O. Gal-Garber, and D. Sklan. 2003b. Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. Poult. Sci. 82:1747–1754.
Vieira, S. L., and E. T. Moran Jr. 1999. Effects of egg of origin and chick post-hatch nutrition on broiler live performance and meat yields. World's Poult. Sci. J. 55:126–142.
Villamor, E., K. Ruijtenbeek, V. Pulgar, J. G. De Mey, and C. E. Blanko. 2002. Vascular reactivity in intrapulmonary arteries of chicken embryos during transition to ex ovo life. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282:R917–R927.
Vince, M. A. 1976. Behaviour and aeration of the respiratory system in the domestic fowl embryo. J. Physiol. 263:465–474.
Waters, N. F. 1935. Certain so-called malpositions: A natural occurrence in the normal development of the chick embryo. Poult. Sci. 14:208–216.
Whittow, G. C., and H. Tarazawa. 1991. The early development of thermoregulation in birds. Physiol. Zool. 64:1371–1390.
Wilson, H. R. 1997. Effects of maternal nutrition on hatchability. Poult. Sci. 76:134–143.
Yoshizaki, N., Y. Ito, H. Hori, H. Saito, and A. Iwasawa. 2002. Absorption, transportation and digestion of egg white in quail embryos. Dev. Growth Differ. 44:11–20.