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IN OVO FEEDING – TECHNOLOGY OF THE FUTURE – A REVIEW

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Abstract

Over the years, due to genetic selection, improvement of the characteristics of laying and meat poultry with less feed consumption per kilogram of body weight gain has been achieved (FCR). As the productivity of poultry increased, the demand of embryos for nutrients changed. However, the chemical composition of an egg has remained practically unchanged, and therefore, it began providing the embryos with suitable substances with the help of *in ovo* technology. Improvements in hatching were achieved through the administration of fructose, sucrose and grape seed extract (GSE), while the weight of a one-day-old chick is affected by the injection of amino acids with glucose and magnesium. In addition, amino acids and carbohydrates applied to an egg have contributed to an increase in the activity of digestive enzymes and maturation of the intestine. In connection with early stimulation of the intestinal tract of broiler chicks, they obtained higher weight gain. Supplementation with vitamins positively affects the increase of birds' immunity and body weight at the end of breeding. On the other hand, the injection of an insulin-like growth factor (IGF-I) influenced the growth and development of muscle tissue during the first weeks of life.

Key words: poultry, *in ovo*, amino acids, carbohydrates, hormones

Over the past 50 years, great progress in improving the functional characteristics of poultry has been achieved through genetic selection. Currently, laying hens, during their 52 weeks of laying abilities, lay more than 320 eggs, and broiler chicks reach 50- to 60-fold higher body weight, counting from hatching to slaughter (Druyan, 2010). From 1957 to 2005, the body weight of broilers increased by over 400% (Figure 1). Zuidhof et al. (2014) analysed genetic selection and its effects and found that the rate of annual increase in the body weight of broiler chicks amounts to 3.30% (42 days of breeding). Moreover, the birds reach a higher body weight over a shorter period of time while consuming less feed per kg of body weight gain. The FCR indicator (Feed Conversion Ratio) has decreased every year on average by 2.55%. In 1957, broilers consumed about 2.85 g of feed per g of body weight, while in 2005 they needed about 1.67 g.

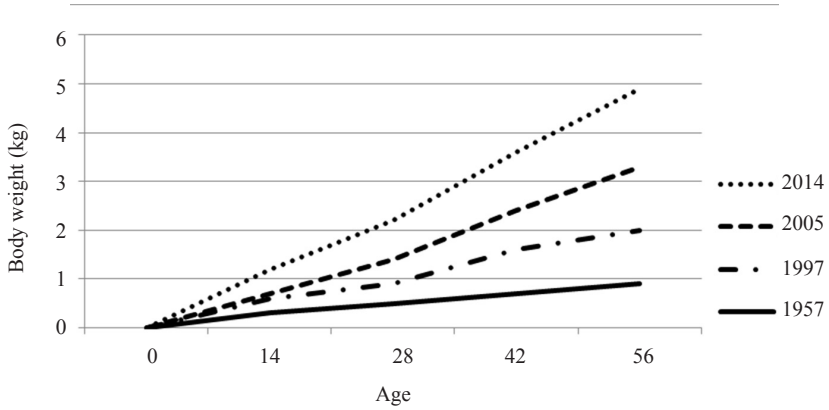

Figure 1. Body weight of both sexes. University of Alberta Meat Control strains unselected since 1957 and 1978, and Ross 308 broilers (2005 and 2014) (Zuidhof et al., 2014)

These results indicate that intense genetic selection has increased the performance of the economically important production characteristics of broilers while contributing to growth of efficiency in the poultry industry. However, the rapid rate of growth and development of birds has caused a number of adverse complications, including ascites, skeletal abnormalities, immunosuppression and increased susceptibility to infectious diseases (Havenstein et al., 2003; Emmerson, 1997). Problems during breeding may be due to different growth and development during embryogenesis and the postembryonic period (Buzała et al., 2015).

Table 1. Amino acid composition of an egg

Amino acid	1945[a]	1950[b]	1998[c]	2011[d]
Arginine	6.4	5.9	-	-
Histidine	2.0	2.41	2.28	-
Lysine	5.2	6.4	6.28	6.28
Phenylalanine	5.8	7.5	9.75	9.57
Tyrosine	4.8	3.75		
Tryptophan	1.4	-	1.48	0.65
Threonine	3.9	4.0	4.72	4.7
Cystine	2.2	-	-	-
Methionine	5.2	5.4	5.96	5.96

[a]Munks et al., 1945; [b]Lewis et al., 1950; [c] Kunachowicz et al., 1998; [d] Wereńska and Okruszek, 2011.

Meanwhile, the chemical composition of an egg has changed over the years (Table 1). The use of *in ovo* feeding with natural nutrients, such as amino acids, carbohydrates, vitamins, stimulants and hormones, can support poultry embryonic development and better prepare poultry chicks for intensive development. Some experiments with the use of *in ovo* feeding technology have shown that the injection of nutrients affects the physiological state of broiler embryos before and after hatching. Proper injection improves not only hatching, but also the nutritional status of the chicks and results in a greater predisposition to growth (Liu et al., 2011; Selim et al., 2012; Ebrahimi et al., 2012).

Methods of *in ovo* technology

The literature mostly specifies that the *in ovo* technology involves injection of various substances into the air chamber or directly into the egg with developing embryo. However, the application site of a given component can vary and mainly depends on the age of the embryo. According to Ebrahimi et al. (2012), in fresh eggs and eggs which are in the initial stage of embryonic development, components are administered to the egg protein at a depth of 12 mm, which allows for the specification of a component as close to the germinal disc as possible. At a later stage of embryonic development, nutrients are usually administered to the yolk sac. The yolk sac is the perfect place for injection of the substance, due to its surface area and ability to digest nutrients. After 17 days of incubation, the yolk sac is resorbed, and a different area of the egg is used in *in ovo* technology – the air chamber and amnion.

In *in ovo* technology, an insulin syringe or devices that are able to inject a larger group of eggs are most commonly used. The needle used in such devices has a length of 18.4 mm and a diameter of 1.27 mm and is designed to be able to reach the amnion (Zhai, 2011).

Amino acids

An egg contains a set of amino acids necessary for the growth and development of the embryo. As shown in Table 1, the amino acid composition of an egg has not changed over the years. On the other hand, the birds' demand for nutrients during the pre- and post-embryonic period has changed (Munks et al., 1945; Lewis et al., 1950; Kunachowicz et al., 1998; Wereńska and Okruszek, 2011). It is essential to provide amino acids when feeding laying hens. As Keshavarz and Jackson's (1992) research shows, laying hens that receive feed poor in amino acids and proteins can lay smaller eggs, which in turn may indirectly contribute to the unmet needs of the amino acids of the embryo during the incubation period, especially in small eggs.

In embryonic development, all amino acids are essential; the absence of any of them causes protein synthesis impairment and a disturbance of the homeostasis of the embryo, which in turn results in impaired growth and development of the young growing organism. This is confirmed in the research by Ohta et al. (2001) conducted on broiler chickens. The authors revealed that administration *in ovo* to the yolk on the 7th day of incubation of the whole set of amino acids suspended in 0.5 ml of sterile water increased the weight of the bird (53.9 g) on the 1st day of life and the chick's weight of the total mass of an egg (79.0%) compared with the control group and the group in which distilled, sterile water was administered (0.5 g and 3.3%, and 0.7 g and 3.1%, respectively). These studies confirm the test results conducted on ducks by Gaafar (2009), where as a result of the injection of amino acids, 6.2 g higher chick weight on the day of hatching and 2.4% higher chick mass ratio to the egg mass was achieved in comparison with the control group. Gaafar et al. (2013), in specifying the set of amino acids (0.5 ml and 0.75 ml), indicated an increase in mass of lymphoid organs. Al-Murrani (1982) also found that injection on the 7th day of the identical amino acid composition as found in the egg has a positive effect on both the weight of a chick and a mature bird at the age of 56 days.

A number of studies were also conducted in which only individual amino acids were administered. Coşkun et al. (2014), who conducted experiments on broiler

embryos, demonstrated the positive impact of DL-methionine supplementation, administered to the amniotic fluid. A higher chick weight to total egg weight was shown (72.7%) in comparison to the control group (70.0%). Kadam et al. (2009) demonstrated the positive impact of injecting threonine (Thr) into the yolk sac. This resulted in a weight gain for the chicks and a higher weight gain of 2.4% compared with the group, where the amino acids were injected into the egg's albumen.

Ohta et al. (1999) suggest that the concentration of amino acids in eggs, such as glycine (Gly) and proline (Pro), does not seem sufficient to support embryonic development in the final phase of incubation. This is confirmed by Bhanja and Mandal's (2005) studies, which demonstrated statistically significant differences in the chicks' body weight between groups in which glycine with proline (Pro Gly+) was injected compared with the control group (47.1 g and 43.7 g, respectively). Birds of this group were characterised by better body weight gain (257.9 g) in the first 3 weeks of breeding compared with the group which was untreated with the injection of amino acids (197.5 g), whilst the injection of isoleucine, leucine and valine (Ile + Leu + Val) to the egg had a significant impact (15.2 g) on body weight gain in the first 7 days of a chick's life (87.8 g).

The supplementation of amino acids, such as arginine and glutamine, may be used for the synthesis of other amino acids. Experiments have shown that embryos given arginine and lysine on the 18th day hatched better, and on the 42nd day, gained greater body weight compared with the control group; the administration of only arginine reduced the chicks' hatching time (Shafey et al., 2014).

Tong and Barbul (2004) claim that arginine is an essential amino acid, whose primary function is to participate in protein synthesis. Moreover, this compound can be converted into glucose and, therefore, is called glycemic acid. Arginine is used by a number of metabolic pathways that produce a variety of biologically active compounds that also contribute to maximising the development potential of an embryo by stimulating the secretion of growth hormones. This is confirmed by tests on quails (*Coturnix coturnix japonica*) conducted by Hazim et al. (2011). After injection of 3 g arginine/100 ml of distilled water into the air cell, they demonstrated an impact on the rate of hatched chicks (91.5%), body weight on the 7th (23.1 g) and 42nd days of life (246.3 g), as well as on body weight gain (217.9 g) and the feed consumption ratio (FCR) (3.7). These values were increased respectively by 10.1%, 4.0 g, 25 g, 22.6 g and a smaller 0.4 (FCR) compared to the control group. In tests conducted on turkeys by Foye et al. (2007), it was stated that *in ovo* feeding with a solution containing 0.7% arginine (Arg) in a 0.4% saline solution contributes to an increase of digestive enzyme activity in the small intestine, produced by the pancreas gland. These experiments showed that chicks treated with arginine in a saline solution had between a 1.7- and 2.2-fold higher increase of sucrose activity, 1.7–2.5-fold increase of maltose activity and 1.8- to 2.8-fold higher leucine aminopeptidase activity on the 25th day of embryogenesis compared to the control group. The activity of the above-mentioned enzymes was further tested on the 14th day of life of the birds, reaching a 3-fold increase in values.

The increased bird body weight gains are due to the ability of the intestines to better digest and absorb food by administering *in ovo* compounds that stimulate the

metabolism of the cells of the gastrointestinal tract. Modulators contributing to bypassing the growth restrictions imposed by the limited function of the intestines by increasing the amount of intestinal disaccharide leucine aminopeptidase activity include, among others, arginine. In the first week after hatching, the chicks are subjected to metabolic and physiological changes. The move to feeding from the external environment and, therefore, faster adaptation of the gastrointestinal tract to an exogenous diet seems necessary for increased survival abilities and growth. Hence, *in ovo* feeding can be a tool to significantly improve the survival rate and hatching abilities of the birds, which in turn will result in an economic effect (Foye et al., 2007).

Carbohydrates

The rapid growth of an embryo is associated with high energy demand. Chicken eggs are rich in proteins and lipids but poor in terms of the carbohydrate content (Burley and Vadehra, 1989). Glucose is stored in embryos mainly in the form of glycogen in the liver and in the glycolytic muscles. The natural resource of carbohydrates is not able to meet the metabolic needs of an embryo, especially during late embryogenesis. In the homeostatic regulation of the glucose level in the blood, an embryo is forced to generate energy using a variety of metabolic processes, such as gluconeogenesis, wherein the substrate is the glycerol or amino acid available from lipolysis and proteolysis (Klasing, 1998). However, at the time of hatching, embryos prefer glucose instead of fatty acids for energy production, since during internal hatching, the availability of oxygen is limited, and with the same amount of consumed oxygen, glucose oxidation provides more energy than the catabolism of lipids. The limited amount of glucose forces an embryo to start the lipolytic and proteolytic processes. Proteolysis is an unfavourable process, as it involves the degradation of proteins, which adversely affect embryo development (Pearce and Brown, 1971).

Due to the increased demand of embryos for glucose during hatching, attempts were made to investigate the effect of the injection of carbohydrates *in ovo* on embryonic and postembryonic development (Table 2). The administration of different types of carbohydrates into the amnion undoubtedly increases the level of available energy for an embryo and reduces energy consumption from the metabolism of proteins and lipids during internal hatching. Zhai et al. (2011) showed that injecting carbohydrates (glucose, sucrose, maltose and dextrin – 0.25 g/ml) significantly increases the body weight of chicks, which increases in direct proportion to the volume of the administered solvent. However, a large amount of administered fluids has a negative influence on the hatching results. The authors suggest that in order to keep a hatching rate of 90% for fertilised eggs, no more than 0.4 ml of fructose and sucrose and 0.7 ml of glucose, maltose and dextrin should be injected (Zhai et al., 2011). The volume of the solvent is important in view of the necessary water loss during embryonic development. A considerable volume of solvent may lead to excessive hydration of the embryo and result in its dying. Research also shows that not all carbohydrates are equally effective. It is recommended not to give fructose, because, compared to other carbohydrates, it lowers the body weight of chicks. Furthermore, in the experimental group in which fructose was administered, lower hatching results were recorded (Zhai et al., 2011).

Table 2. The influence of selected substances administered *in ovo* on embryonic and postembryonic development of embryos

Incubation day	Preparations/Substances	Dose	Place of administration	Effect
3	Hormone: IGF-I (Kocamis, 1998)	100 ng	albumen	- the development of the muscle tissue in male chicks
7	Glucose and magnesium (Salmanzadeh et al., 2012)	Glu (100 mg) + Mg (4 mg)	albumen	- decreased hatchability results - weight gain of chicks
	Amino acid composition identical to the pattern (Ohta and Kidd, 2001)	53 mg/0.5 ml	air cell	- an increase in slaughter productivity and the participation of pectoral muscles in the carcass - an increase of the ratio of chicken weight to egg weight
12	Amino acid composition identical to the pattern (Gaafar, 2009)	0.5 ml	yolk	- improvement in hatchability - an increase in the body weight of a chick and the ratio of chicken weight to the weight of an egg
	Amino acid composition identical to the pattern (Gaafar et al., 2013)	0.5 ml 0.75 ml	yolk	- an increase in lymphoid organ weight
14	*Vitamins: A, E, C, B_1, B_6 (Goel et al., 2013)	A (100 IU), E (0.5 II), C (50 mg), B_1 (100 ug), B_6 (100 ug)	yolk sac	- vitamin B_1 and B_2 improve the growth of birds in breeding - A, B_1, B_6 and E may modulate immunity
	Vitamin E (Salary et al., 2014)	E (15 mg and 30 mg)	yolk sac	- immunity increase
18	Carbohydrates: glucose, fructose, sucrose, maltose and dextrin (Zhai et al., 2011)	0.25 mg	amnion	- weight gain - fructose reduces the body weight of chicks and hatching results
	L-carnitine (Keralapurath et al., 2010)	2.0–8.0 mg	amnion	- prolonged process of hatching - no effect on the results of egg hatching and postembryonic development
	Grape seed extract (GSE) (Hajati, 2014)	4.5 mg	air cell	- weight gain and feed consumption during breeding - a decrease in the *Escherichia coli* population in the ileum

Table 3. Egg hatching results, body weight and tissue composition of 42-day-old broilers (Salmanzadeh et al., 2012)

Treatments	Hatchability (%)	Weight of newly hatched chickens (g)	Body weight (42 days of age) (g)	Carcass (%)	Breast (%)	Leg (%)	Wing (%)	Neck (%)
Control	88.8 a	40.2 d	2160 d	67.3 c	25.6 d	29.8	5.16	6.82
*Sham	74.2 b	40.2 d	2151 d	67.4 c	25.7 d	29.7	5.08	6.87
*Glu (75 mg)	76.8 c	40.6 c	2257 c	68.6 b	26.1 c	30.1	5.21	6.96
Glu (100 mg)	78.9 b	40.9 b	2261 c	68.5 b	26.4 b	30.1	5.19	7.02
*Mg (4 mg)	79.1 b	39.7 e	2148 d	67.2 c	25.6 d	29.7	5.09	6.80
Glu (75 mg) +Mg (4 mg)	74.6 d	40.9 b	2306 b	68.5 b	26.5 b	30.2	5.23	7.40
Glu (100 mg) +Mg (4 mg)	75.1 d	41.9 a	2369 a	68.9 a	26.8 a	30.2	5.24	7.09

Different letters (a, b, c, d or e) show significant difference.
*Sham = injected with 0.5 ml of deionised water, Glu = Glucose, Mg = Magnesium.

The observed increase in the body weight of chicks treated with carbohydrates during embryonic development may be associated with the improved development of the gastrointestinal system of day-old chicks, which was confirmed in the research of Kornasio et al. (2011). It was stated that the effect of the composition administered *in ovo* was long term and resulted in the final weight of the birds. The *in ovo* injection treatment of dextrin and β-hydroxy-β-methylbutyrate-calcium salt for fertilised eggs of meat type hens resulted in improved slaughter efficiency, which was probably related to early stimulation of the gastrointestinal tract. Chicks that received a mixture of substances were found to have an increase of muscle in the post-hatching period by increasing glycogen in the liver and muscles, as well as by the proliferation of satellite cells (Kornasio et al., 2011).

Similar results were obtained in studies conducted on duck embryos. Chen et al. (2009) have shown that the administration of glutamine and carbohydrates (sucrose and maltose) *in ovo*, similarly as in the case of chicken eggs, causes weight gain at the end of breeding. In addition, the administration of these components supported the development of the intestines and an increase of pectoral muscle weight, which is probably due to the saving of protein in pectoral muscles during hatching. The weight of duck pectoral muscles increased 24% on the 25th day of incubation and 15% after hatching when compared with the control group.

Confirmation of the abovementioned tests may be found in the results obtained by Salmanzadeh et al. (2012), who studied the influence of administering *in ovo* glucose and magnesium into the albumen on the 7th day of incubation on hatching results and the postembryonic development of chicks (Table 3). The chicks that received a mixture of glucose with magnesium compared to the control group were characterised by higher body weight on the first day of life and at the end of breeding (42nd day of life). Higher slaughter productivity and the participation of the pectoral muscles in the carcass were found in birds treated with glucose and magnesium during incubation. Moreover, these groups had significantly lower feed intake per kg of body weight gain. The chicks were also characterised by a higher concentration of glucose and magnesium in their blood up until the 21st day of life. However, the results of the conducted experiment revealed that *in ovo* treatment reduced the hatching rate (Salmanzadeh et al., 2012).

However, in studies by Bhanja et al. (2008), no effect of carbohydrates on body weight gain in one-day-old chicks and during breeding was found. However, *in ovo* injection of glucose (50 mg) on the 18th day of incubation affected the level of development of the digestive organs and the biochemical blood profile in day-old chicks, as well as in birds during breeding. On the first day of a chick's life, it was found that the experimental group had a higher level of glucose and protein in the plasma and a higher weight of the liver, glandular stomach (proventriculus) and gizzard (ventriculus), as well as small intestine. On the 10th day of life, the chicks treated with glucose had significantly lowered glucose and uric acid in the plasma and a higher weight of the spleen and small intestine. The authors concluded that *in ovo* administration of glucose resulted in the early development of the intestines, allowing for better utilisation of feed during the first days of a chick's life.

The positive effects of carbohydrates administered *in ovo* on the development of enteritis of turkeys was also found by Bohórquez et al. (2007). Providing lactose-hydrate (1.5 ml, 9% solution) to the amnion on the 22nd day of incubation resulted in the elongation of intestinal villi and an expansion of the surface of the intestine.

Vitamins

As a result of oxidative processes, the embryo mainly acquires energy from the decomposition of lipid yolk. Oxidative processes are accompanied by the production of a large amount of free radicals, which lead to cell damage, especially the degradation of polyunsaturated fatty acids in cell membranes. Vitamins counteract the negative effects of free radicals and thereby protect the embryo against damage (Surai, 2000).

Due to the positive effects of vitamins on the development of embryos, they have begun to be used and their impact in the *in ovo* technology carefully examined. As shown in previously conducted studies, the administration of exogenous vitamins contributes to the growth of birds and can modulate the resistance of broiler chickens. This was confirmed in studies that used vitamins A (105IU), B_1 (18 μg), B_2 (36 μg), B_6 (35 μg) and E (1.4 IU) (Goel et al., 2013). The administered vitamins did not cause any changes in the body weight of day-old chicks, and the impact of vitamins on body weight was observed only at the end of breeding. At 42 days of age, both the males and females that received vitamin B_1 or B_2 exhibited higher weight loss compared to the other groups. Vitamins also affected the size of the organs responsible for the immune response. The relative weight of the bursa of Fabricius was higher in the group of chicks injected with vitamins B_1, B_2 and E, and the thymus weight after administration of vitamins A, B_6 and E on the 42nd day of life and humoral immune response (anti-SRBC HA titer) was better in chicks in which vitamin B_1 was injected.

In the Bhanja et al. (2007) studies, which administered vitamins A (100UI), E (0.5 UI) C (50 mg), B_1 (100 ug) and B_6 (100 g), an impact on hatchability rates was shown. From this group of vitamins, B_6 contributed to improving the percentage of hatched eggs. In the group that received vitamin B_6, hatchability was 81.5% compared to the control group, where it was only 72%. Higher mortality before hatching was recorded in the groups where vitamins E and B_1 were injected. Vitamins C and A only contributed to the weight gain of day-old chicks. However, on the 14th and 28th day of life, greater body weight was found for birds in groups in which vitamins E and B_1 were applied.

The positive effect of vitamins on the final body weight was found in the studies by Selim et al. (2012), which were conducted on duck eggs. Both the females and males were characterised by higher body weight at the end of breeding after the administration of vitamin E (10 mg) and vitamin C (3 mg) on the12th day of hatching. The birds from the experimental group, compared to the control group, gained on average 335 g more body weight. However, these groups were characterised by a higher feed conversion ratio of 0.4 (FCR) on average.

Salary et al. (2014), when studying the interaction of vitamin E on the performance of chickens and the immunological blood parameters of birds after hatching,

showed the influence of this compound on the development of the immune system of chicks during breeding. The injection of vitamin E at a dose of 30 mg resulted in increased resistance to such diseases as avian influenza, infectious bronchitis and fowl pest. In the experimental groups, higher levels of the immunoglobulins IgG, IgM, and IgA were recorded.

Nowaczewski et al. (2012), examining the effects of vitamin C on the hatching of chicken and duck eggs, found that a positive effect of the compound was observed only in the latter birds' eggs. In the case of chicken eggs, the injection of vitamin C did not significantly impact the improvement of hatching. On the other hand, in the duck eggs, better hatching results were obtained in the experimental groups where ascorbic acid was injected (independently of the dose and time of *in ovo* injection). The study showed that the average difference in the hatching of the duck eggs between the experimental and control group was 32.5 percentage points.

Other substances

Due to the wide range of effectiveness of hormones, these substances have begun to be injected through the *in ovo* technique. Most studies have focused on the insulin-like growth factor (IGF-I). This interest in IGF-1 is associated with its broad spectrum of activity. This hormone is responsible for the stimulation of DNA synthesis in over 20 types of cells originating from the endoderm, ectoderm and mesoderm (Van Wyk, 1984). IGF type I stimulates the proliferation and differentiation of muscle cell in chicks during the hatching period (Duclos et al., 1991).

In the conducted tests on chicken eggs, it was proven that the injection of IGF type I on the 3rd day of incubation increases the efficiency of growth and development of the muscle tissue during the first weeks of life. Each egg was injected with 100 ng of IGF-I of human origin. This hormone positively influenced the development of muscle tissue. However, in the male groups, a significantly greater gain in weight (12.3%), breast muscle weight (9.9%) and heart weight (11.4%) was observed. There were no differences between the control group and the group of females (Kocamis, 1998).

The Liu (2011) studies, which were performed on duck eggs, confirmed the effect of IGF-1 on the proliferation of muscle fibres. For this purpose, on the 18th, 21st, 24th and 27th day of hatching, samples of pectoral and leg muscles were collected. The results indicate that the body weight of an embryo and the parameters of the muscle fibres, including muscle fibre diameter (MFD) and number of fibre muscles per unit area, were much greater in the groups in which IGF-1 was administered. Moreover, transcription factors MyoGand MRF4 are at a higher level in the experimental groups compared with the control group. These results indicate that the *in ovo* administration of IGF-1 could mediate in the expression of MyoG and MRF4 and in the induction of myoblast proliferation (Liu et al., 2011).

In the previous studies, IGF-1 was also used to determine its effect on the composition and strength of the tibia and the femur bone of broiler chickens. Based on the conducted measurements, it was concluded that the *in ovo* administration of IGF-1 resulted in prolongation of the tibia and the femur in the male group. The femur bone's fracture load normalised in the weight of birds was greater in the groups

treated with the hormone. In addition, higher levels of hydroxyproline were found in the bones of males after injection of IGF-1 (Kocamis, 2000).

Many other substances that have been used with the *in ovo* technique can be found in the available literature. For example, in Hajati's (2014) studies, grape seed extract (GSE) was administered with the use of the *in ovo* technique. Each egg, on the 18th day of incubation, received an injection of 4.5 mg of GSE, which significantly increased the hatchability. The studies did not find any significant effects of the preparation on the body weight of day-old chicks. However, during breeding, average daily weight gain and feed intake increased. Moreover, the injection of GSE decreased the population of bacteria from the *Escherichia coli* group in the ileum.

Another component administered to the egg was L-carnitine, which belongs to the group of bioactive compounds and plays an important role in embryonic development. It facilitates the transfer of the acyl groups of fatty acids from the yolk to the tissues of the embryo. This compound was applied with the use of *in ovo* technology, due to the fact that chicken embryos have a limited ability to synthesise L-carnitine at the time of incubation. However, the use of L-carnitine in a dose from 0.5 to 8 mg/100 ml of solvent did not significantly influence the postembryonic development of the chicks. This compound negatively influenced embryonic development; an increased dose extended the incubation process and decreased hatchability. A negative impact of L-carnitine on the last stage of hatching was also observed in studies in which this component was administered at the stage of storing eggs. L-carnitine reduced the mortality of embryos only in the first step of incubation. In the later period of hatching, it contributed to the death of embryos. The mechanism causing the reduction of hatchability was not discovered and requires an explanation (Ebrahimi et al., 2012).

Other findings were reported in studies conducted by Keralapurath et al. (2010). In the experimental group, the use of L-carnitine prolonged the process of hatching, and the treatment decreased the hatching rate. No statistically significant differences were found between the control and experimental groups. Moreover, the authors, in examining the influence of L-carnitine on body weight, the quality of meat and the contribution of individual dissection elements in the carcass, stated no significant influence was found of any factor on the above outlined properties.

Summary

The use of *in ovo* technology may lead to significant improvement in the efficiency and profitability of broiler production. Scientific interest in the supplementation of nutrients *in ovo* results from the fact that the introduced substances may help embryos overcome the limitations associated with constant chemical composition of an egg, and they can prepare the chicks for intensive production. It should be noted that the period of incubation of a chicken egg lasts 21 days, which constitutes about 60% of the bird's life. Hence, the course of embryogenesis, and in particular the possibility of its stimulation by *in ovo* feeding, has a critical influence on achieving a greater body weight (over 2.5 kg) during breeding, which currently amounts to 35 days. The presented results relate to works conducted in a laboratory, and a condi-

tion for implementation in poultry practice is the development of a suitable device for automatic *in ovo* injection (Bednarczyk et al., 2011).

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