Effect of *in ovo* inoculation on productive performances and histo-physiological traits in commercial birds

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Abstract— Our fast growing strains of poultry breeds which are genetically altered and need extra nutrients for their growth both in embryonic and growth phase of life (Gao et al., 2107). In ovo feeding of leucin metabolite -hydroxyl-methyl-butyrate had a 45% increase in jejunal villus surface area at hatch in comparison with the controls (Tako et al., 2004). In ovo inoculation is the source of nutrients for the developing embryo, which increase the viability of embryo 90% and decrease the stunted growth after hatching (Al-Daraji et al., 2012). Deficiency of any nutrient in these stages can lead to great productive and economic losses which can be overcome by in ovo technique. Multiple ranges of nutrients with their histological effects on tissues at a specific time of inoculation are mentioned here. This technique should be highlight more and more so it can become a routine farm practice like vaccination in future with latest equipment like Egg Inject Vaccination Automation.

Index Terms— In ovo, lymphoid organs, hatchability, amino acids, villus, growth performance, vitamins.

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INTRODUCTION

The administration of anything in the growing embryo of ova termed as in ovo feeding (Uni et al., 2005). In ovo is firstly reported in the 1980s for the vaccination against marek's disease (Sharma and Burmester, 1982). In case of commercial poultry provision of nutrients at the time of pre-hatching is a most effective and beneficial tool and effect the performance of growth till the final age. Limiting availability of nutrients within the eggs hinders the rapid growth of fast growing strains of commercial birds, which can cause the increase mortality of embryo, poor nutritional status, and decrease in growth performance (Ebrahimi et al., 2017). In the late embryonic stages and immediate to post-hatch production of small intestine mucus occur (Uni and Ferket, 2003). Supply of nutrients to the developing embryo at the age of 18 before hatch, has shown remarkable effects on the enteric development and metabolism, it is regular supply of some very important nutrients during the first few days (3-4 days after hatching).

Near the hatching many changes occur in the physiology and metabolism of embryo. Deficiency may arise from ancestors/maternal diet which can be inherited; these things can be overcome by the provision of in ovo feeding (Wilson et al., 1991). Utilization of amino acids are more during the early phase of incubation like arginine, glycine, proline (Ohta et al., 1999). In ovo inoculation of nutrients into the amnion showed that it improved the development of intestine as it increased the villus height (Tako et al., 2004). Relative weight of visceral organs like liver, proventriculus, gizzards, and small intestine was reported higher in those birds which are inoculated with carbohydrates (Bhanja et al., 2008). In future in ovo inoculation is focused on the quality genes alteration, which will defiantly raise the production (Liu et al., 2012). In ovo technique can become more frequent if it combined with the vaccine, before this it needs more trails like the type of

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inoculating material, combined effect of different nutrients and the time of inoculation (Shariatmadari et al., 2012).

Table No.1. *In ovo* injection of some ingredients and target sites

Author(s)	Species	Injected Substances	Target site
Gao et. al 2017	Chicken	L-arginine	Amnioitic fluid
Zhao et.al 2017	Chicken	Creatine pyruvate	Amnion
Kornasio et.al 2011	Chicken	Dextrin, Ca–β-hydroxy-β-methylbutyrate & NaCl.	Amnion
Shizhao et.al 2015	Chicken	Folic acid	Yolk sac
Lotfi et.al 2013	Chicken	Ghrelin	Albumin
Ohta et.al 1999	Chicken	Amino acid	Yolk & Air cell
T.T. dos et. al 2010	Chicken	maltose, multivitamin ,zinc-glycine, glutamine	Amnion
Gonzales et.al 2013	Chicken	25-hydroxy cholecalciferol	Allantoic cavity
Keralapurath et.al 2010	Chicken	L-carnitine	Amnion
Kadam et.al 2008	Chicken	Threonine	Yolk sac
Kornasio et.al 2011		Dextrin and β-hydroxy-β-methylbutyrate–calcium salt in a saline solution	Amnion
Subrat et.al 2012	Chicken	Lysine, arginine, threonine & methionine	Yolk
Foye et.al 2006	Turkey	β-hydroxy-β-methylbutyrate (HMB), and carbohydrate.	Amnion
Foye et.al 2007	Turkey	Arginine & β-hydroxy-β-methyl-butyrate	Amnion
Foye et.al 2006	Turkey	Arginine And/or ß-Hydroxy-ß-Methyl Butyrate	Amnion
Al-Daraji et.al 2012	Quail	Arginine	Air cell
Zhai et.al 2011	Chicken	Carbohydrates	Amnion

IN OVO EFFECT OF AMINO ACIDS ON METABOLIC AND PHYSIOLOGICAL CHANGES

Time of inoculation is considered as the crucial time for the best result obtains after the hatch (Salahi et al., 2011). Arginine is the main amino acid, include in limiting type of amino acid, and it can activate the various hormonal reactions in the body, and serve as the cell signaling molecule for the growth (Caroll et al., 2016). Development of muscles and growth performance significantly affected by the feeding of arginine in diet, this study also explained the muscles fiber diameter and deposition of protein in muscles (Fernandes et al., 2009). In ovo administration of amino acids to the breeder group not only improves the hatching percentage but also increase the weight of chick at the time of hatching (Ohta et al., 1999). In this study researcher explain the dietary requirement of leucine in broiler with the correlation of activating the mammalian target of rapamycin pathway (Deng et al., 2013). In ovo administration of different nutrients like amino acids, vitamins, fatty acids, and different trace elements in broiler birds studied to enhance the growth parameters (Bakyaraj et al., 2012). Lysine and methionine are the amino acids used in the bird diet and their significant effect on the growth performance and immune responses studied in this research (Bouyeh, 2012). In the in ovo technique different gauge needles used for the inoculating agents, but they found no effect on hatching percentage and the weight of chicks at the time of hatch (Ohta et al., 2002). Availability of amino acid like glutamine in feed just after post hatch within forty eight hour affects the small intestine development and bird performance till the maturity (Yi et al., 2004). 180 of growing male turkeys at the age of 56 day were weighed individually. Dietary treatment

consisted of basal diet supplement with 0, 0.5, and 1 gm arginine/kg. In conclusion arginine increased the serum chemistry, packed cell volume, increased total protein value in serum and reduced the enzymes present in serum when included 0.5 gm arginine/kg. It also increased thymus and spleen relative weight and decrease in salmonella count in small intestine of turkeys (Oso et al., 2017). Amino acid administration during the incubation period into the egg provide significant results after hatching, but the best site of inoculation according to this research is the yolk and the extra-embryonic coelom in the egg (Ohta and Kidd, 2001). Different amino acids are compared for the increase of growth performance in broilers and histomorphometry done for this purpose (Kolaei et al., 2015). Significantly increased effect of lysine and crude protein in broilers diet with help of meta-analysis method explored in this study (Faridi et al., 2015). Inoculation of a combination of amino acids resulted in higher hatchability percentage, higher initial body weight at the time of hatching, and increase in feed intake than un-injected group. Liver weight was higher as compared to non injected group and antibodies titers were similar as in control group (Gaafar et al., 2013). In this study researcher conclude that in ovo injection of silver nano particles either in combination or alone with amino acids was not affect the growth of embryo but it enhanced the immune competence, which is the indication that they are the agents which have the potential for increase of immunity in chicken embryo. Silver nano particles have different in physiochemical characteristics as compared to their large counterparts due to high ratio of surface to volume. It also has antimicrobial property used for water purification. It also has the ability to penetrate in tissue and cells (Bhanja et al., 2015). Injecting the amino acid like arginine influences growing embryo, and improves the post-hatch production performance. It can also be concluded that weight loss during the transportation was due to stress; this can be overcome by the provision of arginine during embryonic development. Broiler farm economy index and broiler feed price ratio were best in those groups fed in ovo (Nayak et al., 2016). In this research L-carnitine used as a feed additive & its significant effect seen on metabolism (Arslan, 2006). L-carnitine inoculation in eggs can significantly affect the liver glucose level, moisture level concentration in different body muscles, and biochemical nutrient profile of body organs (Keralapurath et al., 2010).

EFFECT OF *IN OVO* INOCULATION ON HATCHABILITY PERCENTAGE AND INTESTINAL MUCOSA

In this study it is evaluated as, 0.05 ml inoculation of threonine improved the intestinal morphology which includes height and width of villus, muscular layer thickness of jejunum, and goblet cells of chicks. Quantity of goblet cells increased in each villus which is observed as increased mucin secretion. Villus length to crypt proportion is the indicator for higher of enterocyte maturity which increased the functional capacity, in other words it increased the area of absorption of nutrients (Kermanshahi et al., 2107). Availability of carbohydrates in a specific proportion can improve the hatchability, egg sizes, and weight of the yolk sac (Zhai and Peebles, 2011). Hatchability percentage and carcass quality can be improved by L-carnitine inoculation in the broiler eggs, this also affect the carcass yield and quality (Keralapurath et al., 2010). Creatine pyruvate administration via in ovo significantly affects the growth performance, hatchability ($P<0.05$), and energy metabolism in the developing embryo (Zhao et al., 2017). At 20th day of incubation in case of ducks administration of vitamin-C causes increase in hatchability rate by increasing the viability of embryo (Nowaczewski et al., 2012). In ovo effect of aromatase inhibitor, garlic and tomato extract studied and find that there is no significant increase in the hatchability rate, gain in body weight ($P >0.05$) observed in chicken birds (Fazli et al., 2015). Changes in the morphology of intestine mucosa studied up to 12 days of age after post hatch, but most observed changes in the enterocytes observed within 24 hour after hatch (Geyra et al., 2001). Carbohydrates which are less in molecular weight having the ability to develop the gut and increase the absorption capacity and overall growth performance of birds when provided to newly hatched chicks (Bhuiyan et al., 2011). Developments of gut and gut associated lymphoid tissue are combined effect due to delayed in the feeding after post hatch (Shira and Friedman, 2005). Delay in feeding should not accede more than sixty hour after post hatching; otherwise it will decrease the visceral weight and growth performance till the final age (Yang et al., 2009). Secretion of some pancreatic enzymes increased due to early provision of feed after post hatch and absorption also affect by the availability of sodium in feed in post hatch period (Sklan and Noy, 2000).There are the many factors affect the hatchability or viability of embryo during the incubation periods which are discussed in this research

(King et al., 2011). This study investigate the in ovo effect of B-haydroxy and B-methyl butyrate, egg protein an carbohydrates on the glycogen reserves of body at the early age of the chicks (Foyeet al., 2006). Injection of zinc & methionine into the amnion of the egg at 17th day of incubation significantly improves the development of intestine and its functional capacity (Takoet al., 2005). In ovo of arginine and B-haydroxy and B-methyl butyrate significantly affect the production of digestive enzymes and uptake of nutrients during the incubation period and growth performance of the birds after hatch (Foye et al., 2007). Development of gastrointestinal tract and production of goblet cells occur in late embryonic period before hatching and immediately after hatching, so inoculation of carbohydrates at that time cause significantly improvement in the intestinal development and goblet cells (Smirnov et al., 2006). In turkey birds administration of dextrin-iodinated casein solution at 25 days of incubation significantly improved the hatchability and growth performance (Bottje et al., 2010). This study conducted to reveal the structural modifications of digestive (enteric) development of epithelium in the embryos and poults of turkeys. In this study morphological and ultra structural development of intestine (jejunum) mucosa from the 15 day of incubation to 12 day of post hatch age was demonstrated. A marked rise in villus height and depth observed epithelia cells and their epical ends become tight by their junctions and goblet cells started to appeared on the epical ends of villi. Villus morphology becomes changed to finger like projection to leaf like projections. Development of gut occurred during the period of incubation, but the appearance of villi started only at the 2nd half of incubation. Intestinal villi originate from folds of mucosa, which are known as pre-villus ridges (Bohorquez et al., 2011). The present experiment was performed to estimate the impact of in ovo administration of butyric acid of half ml at the day of 7 of incubation, hatchability percentage, gain in body weight; intake of feed and feed conversion ratio were studied in this trail. Results of this study revealed that weight of newly arrived chicks was significantly improved than control group. Poults showed better FCR and gain in weight which was in ovo fed compared to control. In ovo inoculated groups also showed increase in villus height & width for duodenum, jejunum and ileum, so the growing progress also improved in injected groups, which revealed the importance of injected groups (Salmazaidi et al., 2015).

IN OVO INOCULATION EFFECT ON IMMUNE ORGANS GROWTH AND IMMUNE BARRIER OF INTESTINE

Currently it is studied as is facing the problem of delayed access of feed in 24-72 hours after hatching which impact badly on its nutritional status as well as its muscles protein deposition, due to the under development of its GIT and immune organs leads to less growth rate, and also susceptible to infections in early ages of life. So immediate nutrition is required as early as after hatching, this aspect can be covered by provision of in ovo nutrition (Gao et al., 2017). Feeding of embryo during the incubation period and immediate post hatch feeding is the critical period for birds, which can improve the gut development, immune system, carcass quality, hatching weight and epigenetic of the birds (Noy and Uni, 2010). Limiting amino acids are compared with the in ovo technique, which are significantly affect the immune system and development of gastrointestinal tract after hatching till the final weight of the birds (Bhanja et al., 2012). Administration of vitamin-E within the embryo during the incubation period significantly improved the immune system, level antibodies and their humoral responses (Gore and Qureshi, 1997). The above treatment also activated the germinal centers formation in spleen, indicated the enhanced B-cell proliferation in secondary lymphatic organs. This study also revealed that effect is more pronounced after synbiotic treatment than prebiotic treated group. There was a significant age dependent increase in spleen and bursa observed in histological findings, this is due increase in migration of lymphocytes to the spleen and proliferated there, compared to control groups. Increased in bursa weight observed in between day 1 and 21 day in control group and decreased on 35 day, in conclusion the tested prebiotic and synbiotic modified the development of immune system in chicken embryo (Madej et al., 2015). Different levels of lysine are compared for the significant increase in the immune response and antibodies production in birds (Mulyantini et al., 2014). There is a comparison of nutritional alterations in diet and in ovoon the humoral responses toward the diseases (Kidd et al., 2004). Thymus produce the T-cells within the mammalian and avian body, in this experiment researcher studied how the presence of different level glucose may alter the growth and survival of thymus cells within the birds (Bhattacharya et al., 2007). Threonine inoculation in the eggs affects the immune responses, digestive enzymes

activity, and post hatch growth of broiler birds (Kadam et al., 2008).

EFFECT OF *IN OVO* FEEDING ON STEM CELLS

Satellite cell activation by in ovo feeding in the ducks which cause increase in muscle fiber diameter, cross sectional area and ultimately the growth of muscles (Liu et al.,2012).

IN OVO INOCULATION EFFECT ON GENE EXPRESSION

Immune system plays a key role in the progress and growing ability of birds because under development of immune organs and incompetency of digestive mucosa of new hatched out chicks are susceptible to feed antigens of all kinds including bacteria, virus and others. In ovo inoculation provide the better nutritional status, hatching percentage, immune system and finally the better growth performance, so the in ovo administration of threonine cause increased in synthesis of Immunoglobulins and mucin2 gene expression (Kermanshahi et al.,2017). Folic acid requirement during the incubation period can meet by providing it by in ovo; it can also affect the performance of growth, metabolism of folate, and epigenetics regulation of immune genes (Li et al., 2016). Quality genes of growth may be effect by the different nutritional supplement investigated in this experiment (Kita et al., 2002). Arginine is the important amino acid which potent stimulator of creatine, urea and nitric oxide, these are the cell signaling molecules and active the mTOR pathways and involve in protein synthesis (Ham et al., 2014). mTOR cell signaling pathways are activated by injecting the arginine in the developing embryo of human and pig which enhance the viability of embryo (Kong et al., 2012). This study also revealed that present of carbohydrates in lumen of intestine of developing embryo, stimulate the proliferation of mucin production by goblet cells. It is also reported that insulin like growth factor 1& 2 & their gene expressions increased during late phase of embryonic growth (Kadam et al., 2013).

IN OVO SUPPLEMENTATION& ITS EFFECT ON THE GROWTH PERFORMANCE

The present study was performed to evaluate the impact of in ovo administration of glutamine on the hatchability percentage, progress of GIT, progress of growth and the carcass yields characters studied in broiler chicken, findings have demonstrated that there was increased height & width of villus and depth of crypts till the final growth (42 days). In this study different plant extracts inclusion in the diet of birds were compared and their effect on the growth performance was noticed (Hernandez et al., 2004). In addition to all these relative weight of following also increased which was weight of breast, carcass weight; thigh muscles were markedly increased and significantly improved heart, liver abdominal fat, spleen, pancreas and intestine. This data also suggested that it also improved the absorptive capacity of small intestine (jejunum) resulted in great performance in broilers. (Salmanzadeh et al., 2016). Different feeding plans in broilers in different feeding stages & groups studied in this research having effective outcomes (Shariatmadari et al., 2012). Effect of injected methionine in the yolk sac and histomophometry of chicken embryo was investigated. They observed the change in length & width of villus and the crypts depth, with enhanced absorption. Much advancement occur to increase the growth of broiler, due to antibiotic resistance as well as demand of antibiotic free products, they used alternative of antibiotics to enhance the growth. Methionine is one of the non antibiotic alternatives which increased the growth rate provided in diet and in ovo (Mohammadrezaei et al., 2015). The trail was conducted to investigate the combined impact of arginine & threonine on growing performance, GIT, daily intake of feed and final body weight measured at the end of the trial, height of villus and depth of crypts, weight of small intestine, significant changes were seen. At the age of 11, when in ovo inoculation was done, it decreased villus height in duodenum, crypts depth, and villus/crypts ratio remained unaffected in the trail (Tahmasebi and Toghyani, 2015). Different nutrient with their growth enhancement effects injected via in ovo discussed in this research (Lokesha et al., 2017). Feeding of Probiotics in the feed improves the activity of microflora present in ceca which result in improves the growth performance (Mountzouris et al., 2007). In ovo technique and his correlation with growth performance and carcass yield discussed in this research (Wilkinson and Scott, 2006). Intestinal absorptive capacity is directly related with the biochemical and structural changes occur during its development occur in growth (Croom et al., 1999). Delay feeding during the early post hatch period cause underdevelopment of intestine and reduce the muscles growth which results in stunted growth of broiler birds (Bigot et al., 2003). Inoculation of L-carnitine significantly increased the growth performance, glycogen reserves in the muscles and the plasma IGF-1 in the broiler birds (Shafey et al., 2010). Inoculation of different nutrients into the egg at best time of

incubation for the specific inoculating material can give best result for both egg production and growth performance in birds (Dos et al., 2010). B-haydroxy and B-methyl butyrate significantly affect the insulin like growth factor, energy metabolism and growth performance in turkey birds by injecting into amnion layer (Foye et al., 2006). Administration of antibiotics within the bird egg protect the chicks to the infections occurred after the hatch and its effect on growth performance (McReynolds et al., 2000). Development of body muscles by in ovo feeding and the temperature of incubation period affect the growth of body muscles before hatching and growth performance after hatching (Chen et al., 2013). In this study researcher investigated that tryptophan in diet needed for the development and the growth performance of birds from first day till the final age of growth (Corzo et al., 2005).

IN OVO EFFECT OF HORMONES

Exogenous material of ghrelin feeding via in ovo improves the weight of chick at the time of hatching, weight of the muscles and the growth performance of the broiler birds (Lotfi et al., 2013). Testosterone effect of in ovo on the viability of embryo and muscles growth studied in this research with the consideration of gender in broilers (Henry and Burkey, 1999). Thyroid hormone controls the different metabolic pathways in the body, so it plays a critical role in controlling the metabolism and growth performance in the birds (Decuypere et al., 2005). In ovo inoculation of growth hormone increase the growth performance in the birds used for meat purpose, but this improvement depends on the gender (Kocamis et al., 1999). Aim of this study was to fed L-arginine via in ovo cause significantly increase in embryo weight, pectoral muscle mass, gain in body weight, feed conversion but there was no effect on embryo length was observed. Increased in body weight of embryo was due to more development of body muscles, so at the time of hatching there was increase in initial body weight. Growth hormone level in embryonic phase determines the cell proliferation (Azhar et al., 2016).

IN OVO EFFECT OF VITAMINS

Supplement like multivitamins, zinc-glycine glutamine via in ovo injection has beneficial effect on carcass quality, egg size, hatching percentage and overall performance of birds (Dos et al., 2010). Vitamin D3 supplementation via in ovo administration just before hatching has showed beneficial effect on embryo and post hatch development of broiler chicks (Gonzales et al., 2013). By the injection of vitamin-E into eggs there is no change in the performance of birds found but significantly increase in the immune response and hatchability percentage was noticed in this research (Salary et al., 2014). Vitamins are the cofactors for many metabolizing reactions in the body, so vitamin-A and vitamin-C gave the significant effect when provided in ovo, while vitamin-E and vitamin-B1 required just after hatching for the better growth performance (Bhanja et al., 2007).

EFFECT OF *IN OVO* FEEDING ON BONE MINERALIZATION

This is a study of commercially available diluents containing micro-minerals as a supplement was examined (Mn, Zn, & Cu) on hatchability percentage and quality of chicks in broilers. These mineral when injected separately gave negative effect on hatchability, and had no effect like that when injected separately. In early post-hatch period minerals reserves are essential for proper development, based on this information we conclude that mineral enriched solutions has the capacity to improve mineralization, so inoculation of mineral solutions effect the performance of growth, development of bones and their mineralization in broilers (Oliveira et al., 2015).In ovo feeding of insulin growth factor significantly improved the mechanical properties of bone (Kocamis et al., 2000). Growth performance of broiler and bone mineralization can improve while providing the L-arginine via in ovo inoculation (Sanami et al., 2014).

IN OVO SUPPLEMENTATION IN LATE STAGE OF INCUBATION

During the incubation period optimal amount of amino acids should be accessible to fulfill the increasing demand of functional & metabolic processes, especially in the late stage embryonic development. Metabolism of carbohydrates & proteins in the 1st two week of incubation is very important to provide energy for the growth of tissue (Shafey et al., 2014). Provision of in ovo feeding is the solution to compensate depleted requirement of embryo during the incubation period at a specific time for the required ingredient (Goncalves et al., 2013). Delayed feeding after hatching causes the stunted growth and utilization of reserves from the muscles, which cause poor development of muscles resulting poor final growth (Kornasio et al., 2011). At 23rd of incubation in the ducks glutamine and carbohydrates are injected via in ovo method, this inoculation significantly

improved the small intestinal development and pectoral muscles growth in ducks (Chen et al., 2009). Importance of early nutrition including the in ovo feeding and its comparison with the delayed feeding studied and explored in this research (Willemsen et al., 2010). L-carnitine injected 2.00 umol into the fertilized eggs of broiler at 17 day of incubation age significantly enhanced the hatchability percentage (Zhai et al., 2008). Early nutrition is the key to improve the growth performance and development of organs immediately after the hatch (Lilburn et al., 1998). Administration of carbohydrates (0.1, 0.4, 0.7 & 1 ml) via in ovo at the age of 18 day of incubation significantly improved the viability of embryo which leads to increase in the hatchability percentage (Zhai et al., 2011). Delayed feeding and in ovo feeding groups are compared for retarded growth, gut development, embryo viability and glycogen reserves of liver in this study (Kornasio et al., 2011). Immediate post hatch feeding to the chicks showed significant positive effect on the growth performance and meat quality and reduce the detrimental effects like stunted growth on the birds (Henderson et al., 2008). In turkey birds delayed feeding impact stunted growth results due the under development of gastro intestinal tract and damage to the enterocytes (Potturi et al., 2005). Enzymatic activity of embryo greatly changes at the day of hatching which greatly influence absorption of nutrients from digestive system. At the first four day of age after hatching absorption of fatty acids, carbohydrates and proteins showed high. The best technique which is studied is the in ovo for the transferring of nutrients from broiler breeders to their eggs. In ovo administration at age of 0 and 7 day has the beneficial effect on embryonic development and post hatching performance. L-carnitine in ovo feeding at the age of 17 and 18th of incubation was not beneficial; in ovo inoculation of carbohydrates upgraded the status of energy in the late stages of embryo and upgraded as well the growing progress of broiler chicken (Cardeal et al., 2015).

CONCLUSION

In ovo is the emerging technique for nutritionist to meet the high demand of growing embryo, and to produce high quality carcass yield. This technique also many benefits like decrease post hatch mortality rates, morbidity rates, grater efficacy to utilize dietary nutrients, improved immune status and GIT antigens, reduction in the development of skeletal muscles disorders, improved and increased body muscles and yield of carcass. Injected groups also have increase villus surface area, density of villi also increased and higher gene expression/epigenetic.

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