

## In-Ovo Feeding: Jumpstarting the Development of the Chick

### Case Study 8

Research goal	To determine the effect of in ovo feeding on growth performance and economically valuable production traits of broiler and turkey flocks.
Beneficiaries	Poultry industry and poultry integration, Consumers
Activities conducted in order to achieve the objectives	<p>The research demonstrated that supplementing the chick embryo amnion with nutrients enhances neonatal development. Optimal feed formulation, timing and delivery technique, pH and osmolality limits and cost-efficacy of the formula ingredients that were studied.</p> <p>Transcriptome analysis provided evidence of enhanced expression of nutrient transporters following in-ovo feeding.</p>
Funding	BARD awards: IS-3311-02, US-4113-08 and US-5074-18CR; \$866,000. Industry: \$1,600,000
Publications	13 journal publications, of which 6 are in the top impact factor quartile (Q1) and 2 have over 100 citations
Students involved	5 Ph. D students were involved in the BARD supported research. Current positions: 1 is in academia in the US, 1 holds a research position in the USDA, ARS working on human nutrition for at-risk populations and 3 work in agri/biotech industries in Israel
Stakeholders' collaboration	The new science of neonatal chick nutrition provided a platform for nutritionists to optimize poultry production. More than 15 research groups in industry and academy are using in ovo feeding and in ovo stimulant delivery.
Environmental impact	none
Social impact	Injection of probiotics could reduce antibiotic use on days following hatching
Commercial engagement	Embrex, Cargill, Nutrico, Zinpro,
Patents	2 patents joint to N. Carolina and Hebrew University
Practical agricultural applications	In-ovo injection of feed on days 17–18 of egg incubation, which contributes to their post-hatching performance and development. Injection can be conducted with existing in ovo vaccination systems
Economic impact	Innovation not yet adopted. No monetary benefit calculated.

## 1 Objective: To Determine the Effect of In-ovo Feeding on Growth Performance of Chick Embryos

Nutritional and physiological limitations during the ‘window of hatch’ period - from when a chick hatches until its first feed - lead to problems in hatchling quality, in microflora establishment and lead to variation in growth and in body weight uniformity of broilers. The physical and nutritional properties of in-ovo feeding (IOF), that is feeding of the chick embryo, was studied in order to determine the effect of IOF on growth performance and economically valuable production traits of broiler and turkey flocks.

## 2 Research Activities

Between 2002 to 2018 3 BARD awards (IS-3311-02, US-4113-08 and US-5074-18CR) were granted to Zehava Uni (Hebrew University), Peter R. Ferket (North Carolina State University) and Eric. A Wong (Virginia Tech) for research on in ovo feeding of broilers and on composition of the egg yolk nutrients during incubation. See Appendix A for full details of the awards.

In the first award, IS-3311-02, Z. Uni and P. Ferket demonstrated that injecting nutrients into the amnion during incubation (3-4 days before hatch) enhances neonatal development of the chick. It is a generally accepted practice throughout most of the industry that the newly hatched chicks have no access to feed or water. The in ovo feed supplies the embryo with additional nutrients prior to hatching and those nutrients continue to be utilized by the chick post-hatch during this fasting period. Post-hatch performance is enhanced by increasing body weight and post hatch muscle growth, maturing the intestine by enhancing villi growth, and improving glycogen deposition in the liver. The In-ovo feeding gives chicks more energy when hatching and in their first hours of life. As a result, right after hatch in-ovo-fed birds are more active and eat more feed leading to increased breast meat yield, growth rates and higher feed conversion ratios. In ovo feeding was shown to reduce post-hatch mortality and morbidity related to dehydration and starvation. This first and pioneering research focused on optimization of the feed formulation and delivery.

The next BARD award of Z. Uni together with E. A Wong (US-4113-08) studied nutrition of the developing embryo. The research included (i) transcriptome analysis to study the expression profiles of nutrient transporters and digestive enzymes in the yolk sac membrane (YSM) throughout incubation, (ii) study of the pathway by which the uptake (absorption, digestion and transportation) of nutrients is mediated and (iii) the effect of amnion enrichment of the embryo by in ovo feeding of minerals and other nutrients on skeletal development and skeletal properties throughout the broilers growing period.

The ongoing study of Z. Uni and E. A Wong (US-5074-18CR) links between the in-ovo feed research outcomes and the knowledge gained on the role of the yolk sac in the mediation and transport of nutrients to the embryo for optimal chick development. The

research investigates in-ovo feed ingredients (nutrients and probiotics) that will upregulate the number of cells expressing nutrient transporters and increase host defense peptides to resist intestine pathogens.

### 3 Academic Impact

#### 3.1 Publications

13 peer-reviewed journal publications have been published based on research from the 2 early BARD awards. Of these, 6 are in the top impact factor quartile (Q1) and 2 have been cited over 100 times.

#### 3.2 Capacity Building

Five Ph. D students were involved in the BARD supported research. Current positions: one is in academia in the US, 1 holds a research position in the USDA, ARS working on human nutrition for at-risk populations and 3 work in agri/biotech industries in Israel

Two of the Ph. D students were awarded BARD post-doctoral fellowships for research with US groups in 2013 and 2014.

#### 3.3 Stakeholder's Collaboration

P. Ferket is based in N. Carolina and the proximity of the Research Triangle Park (RTP) was extremely advantageous to this project. In-ovo injection was conducted both by in-lab injection, as well as with the use of commercial in-ovo vaccinations systems in collaboration with the RTP based company, Embrex. The Embrex Inovoject<sup>®</sup> vaccine-delivery system is the industry standard for in ovo technology.

The research on in-ovo feeding by the BARD researchers established a new science of neonatal chick nutrition. This research has provided opportunities for nutritionists to optimize poultry production. More than 15 research groups in industry and academia are using the in ovo feeding concept established during the BARD awards.

The work conducted by these research groups has led to the publication of more than 200 papers and abstracts on in-ovo feed and stimulants in scientific journals and conferences. On average, 2-3 peer reviewed papers are published on this topic in scientific journals every month.

These research groups around the world have continued to study nutrient injection of amino acids, carbohydrates, trace minerals, vitamins and fatty acids. The research has shown many positive outcomes; such as faster growth and higher final weights, enhanced expression of nutrient transporters, support of bone development and advanced intestine development and digestive capacity. Furthermore, *in ovo* injection of various biologics that may benefit the future health of the chicken (and not only supply the chick embryo with

additional nutrients that promote improved growth) have also been studied. Such compounds include various immunostimulants, antibodies, live beneficial bacteria, prebiotics, and synbiotics.

The BARD researchers have collaborated with academic groups from Europe, Brazil and Australia.

## 4 Commercial Engagement

Between 2005-2007 the Israel-US Bi-national Industrial Research and Development Foundation (BIRD) awarded \$1,600,000 to Avian Tech Ltd. (a Hebrew University-Yisum company) and Embrex Inc for a collaborative academic and industry research study entitled “Improve broiler production efficiency by developing in-ovo nutrients for mass delivery to broiler chickens”. The Embrex Inovoject<sup>®</sup> System can inject up to 70,000 egg per hour. Profs. Uni and Ferket acted as consultants for and were part of the ATL research team.

Additional industrial partners with whom negotiations for licensing of the innovative feeding procedure have been conducted are: Cargill, Nutrico and Zinpro Performance Minerals.

### 4.1 Patents

*Enhancement of development of oviparous species by in ovo feeding, Uni Z and Ferket P.R.*, US6,569,878, Granted 15-7-200 to Hebrew University and North Carolina State University.

*Enhancement of development of oviparous species by in ovo feeding of enteric modulators, Uni Z and Ferket P.R.*, US8,734,837 B2, Granted 27-5-2014 to Hebrew University and North Carolina State University

## 5 Practical Agricultural Applications

Chicks are hatched over a wide time window, and it is not uncommon for some chicks to be without feed and water for 2–3 days before they are transferred to the farm. In-ovo fed hatched chicks have proven to be more resilient and robust, which enhances to their post-hatch development. The nutrient balance and key metabolic co-factors of the amnion meal can be modified and influence subsequent phenotypic traits of economic importance for the poultry industry, making the perinatal feed methodology extremely appealing to the industry. Moreover, the period of embryonic and neonatal development is approaching 50% of the productive life of modern broilers and turkeys, and this early period is thus considered crucial for the lifetime performance of the chick.

Despite all the positive effects observed in in ovo-fed birds, the method has not yet been widely adopted by the poultry industry. One early barrier was the purchase in 2006 of

Embrex, by Pfizer Animal Health (now Zoetis). Zoetis strategically focused on vaccinations and discontinued their collaboration with the researchers on use of their Inovoject® vaccine-delivery system for nutrition.

On a practical level adoption is feasible. Automated in ovo feeding protocols can be adapted to injection machines at the hatchery. The in ovo feeding formulations consist of GRAS (generally recognized as safe) materials, considered as feed additives requiring minimal regulatory compliance and the costs of IOF ingredient formulation, estimated at 1-3 cent per ten doses, is not a limitation.

### 5.1 Future prospects of in-ovo feeding

The methodology is an emerging field, and research associated with both in-ovo feed and in-ovo stimulation are becoming more prolific and established. A number of factors described below suggest that industry will move towards adopting the methodology.

1) Breeding programs currently focus on improving feed conversion ratio (FCR), that is the kg feed an animal uses to produce a kg of product (rather than being concerned primarily with how to maximize the poultry body weight). This is due to the increasing cost for feed, which accounts for 70% of the total cost in poultry production. In-ovo feed improves FCR by ~ 1 points (10 grams of feed less per kg meat).

2) Growth performance and meat yield of commercial broilers and turkey has improved linearly by about 1% each year, and 85% of this improvement is attributed to genetics and 15% to nutrition. Although genetic selection dictates the genes that are inherited by progeny, there is awareness today that nutrition and management may influence how those inherited genes are expressed. Many of the recent studies focus on the use of in-ovo feed and stimulations to enhance tolerance to immunological, environmental or oxidative stress and metabolic disorders by epigenetic programming.

3) Some global markets (e.g., the EU) have withdrawn the use of antibiotic growth promoters (sub-therapeutic levels of antibiotics) for poultry. Poultry producers are thus facing the challenge of compensating for lost product and profit *via* alternative routes.

4) Poultry nutrition is moving towards higher standards, and parameters such as economic sustainability, consumer confidence and food security are dictating the market. Programmed in ovo nutrition increases production efficiency that is so important to poultry producers, but there is also evidence that it improves the meat quality consumers demand. Broilers that have been raised on a programmed nutrition strategy have reduced carcass fat and produce breast meat that has more appealing color, less drip losses during storage, improved oxidative stability, and lower cooking losses.

## 6 Social Impact

With current management procedures, broilers are administered antibiotics immediately on hatching. Results from in-ovo nutrition trials shows that the practice can help reduce antibiotic use in the first days of a chick's life.

## 7 Economic Impact

### 7.1 Investment Cost

BARD contributed \$866,000 in research funds between 2002-2018. Additional academic funds contributed.

### 7.2 The Benefits

The method has not yet been commercialized by the poultry industry. Some barriers to adoption are now being overcome but to date, we have not included an economic benefit analysis.

## 8 Appendix A: BARD Awards

Table 1: Details of the BARD awards

Project No	Full Title				
	Investigators	Institutes	Budget	Duration	Start Year
IS-3311-02	Enhancement of development of broilers and poults by in ovo feeding				
	Uni, Z. Ferket, P.R.	Hebrew U N Carolina St. U	\$266,000	3 years	2002
US-4113-08	Nutrition of the Developing Chick Embryo: Nutrient Uptake Systems of the Yolk Sac Membrane and Embryonic Intestine				
	Wong, E.A. Uni, Z.	Virginia Tech Hebrew U	\$300,000	3 year	2008
US-5074-18CR	Modulating intestinal cellular maturation and differentiation in broilers by in ovo feeding				
	Wong, E.A. Uni, Z.	Virginia Tech Hebrew U	\$300,000	3 year	2018

## 9 Appendix B: Information providers: Personal communication

- Zehava Uni - PI for BARD grants, Animal Sciences, Hebrew University
- Peter Ferket - PI for BARD grants, Poultry Science, Nutrition, and Biotechnology, Northern Carolina State University