The Intra-Amniotic Administration (*Gallus gallus*) - An Emerging *in vivo* Approach to Assess Bioactive Compounds with Potential Nutritional Benefits

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Background

The broiler chicken (*Gallus gallus*) is a well studied animal model of human nutrition (1,2) to assess the physiological effects of various nutritional conditions. *Gallus gallus* is useful because of its growth rate, anatomy, size, and low cost. This animal is fast-growing and sensitive to dietary deficiencies of trace minerals, such as Fe (3). Figure 1 shows the organs of *Gallus gallus* and the Fe status indicators associated with each organ that are utilized in various studies. Similar to humans, the duodenum is the primary Fe absorption site in the *Gallus gallus* model (3). Fe status of *Gallus gallus* can be determined from the expression of Fe transport proteins in the duodenum, such as divalent transporter 1 (DMT1; the Fe uptake transporter), duodenal cytochrome B (DcytB; reduces Fe at brush border membrane), and ferroportin (a protein involved in Fe transport across the enterocyte) [2]. The functionality of the small intestine, specifically in the brush border membrane (BBM) indicates on food hydrolysis and nutrient uptake which affects the nutritional status of *Gallus gallus* (4) . In the chicken embryo, the bacterial analysis of the caecal content in *Gallus gallus* is used to assess the effects of intra-amniotic administration of prebiotics on the gut microbiome population and its impacts on the nutritional status of *Gallus gallus*. Figure 2 shows the *Gallus gallus* embryonic development.

Figure 2. *Gallus gallus* embryonic development

Figure 1. The digestive tract of *Gallus gallus* and the Fe status indicators associated with the organ

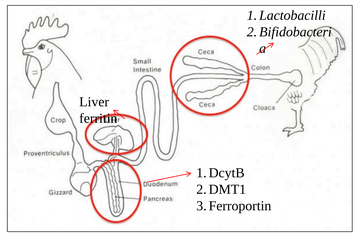
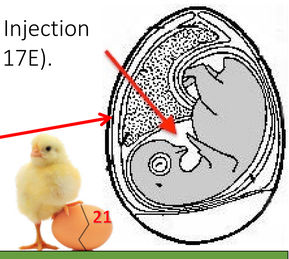


Figure 3. Injection site (day 17E).



Intra-amniotic administration of prebiotics (*Cicer arietinum* and *Lens culinaris*) and duck egg white peptides affects the calcium status and intestinal functionality (5)

Objective: This study aimed to assess the effect of prebiotics from chickpeas, lentils and duck egg whites *in vivo* (*Gallus gallus*).

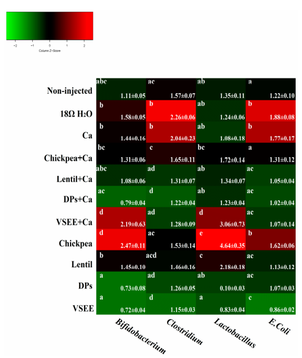


Figure 1. Genera and species level bacterial populations from cecal contents measured on day of hatch. Mean values within genes tested with unlike letters were significantly different ($p < 0.05$).

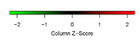
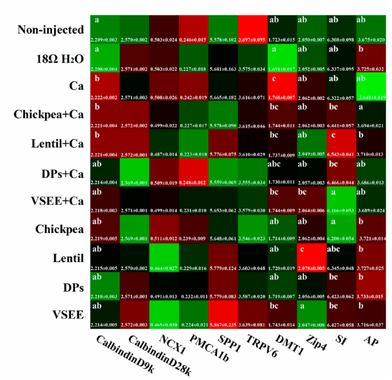


Figure 2. Duodenal mRNA abundance of Fe and Calcium genes in *Gallus gallus*. Mean values within genes tested with unlike letters were significantly different ($p < 0.05$).

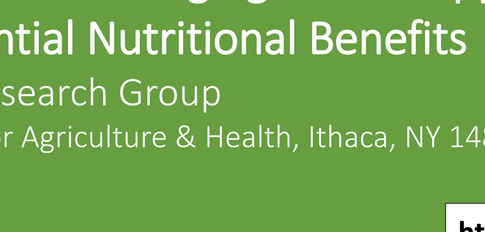


Conclusion: This study demonstrated that the intra-amniotic administration of chickpea and lentil prebiotic extracts and duck egg white peptides may improve calcium bioavailability by the promotion of beneficial populations (*Bifidobacterium* and *Lactobacillus*) and limitation of potentially pathogenic bacterial populations. Moreover, the expression of BBM functional genes was affected by lentil prebiotic extracts. DPs and VSEE may promote and contribute to calcium uptake.

Intra-amniotic administration (*Gallus* gallus) of Nicotianamine and *Triticum aestivum L. prebiotics* affects irons status, hypertension and intestinal functionality (6)

Objective: This study aimed to assess the effect of prebiotics from biofortified wheat and nicotianamine on Fe bioavailability *in vitro* (Caco-2 cells) and *in vivo* (*Gallus gallus*).

Figure 1. Genera and species level bacterial populations from cecal contents in *Gallus gallus*. Mean values within genera tested with unlike letters were significantly different ($p < 0.05$)



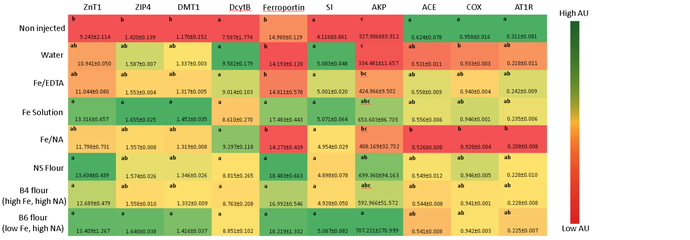


Figure 2. Duodenum mRNA abundance of various genes in *Gallus gallus*. Mean values within genes tested with unlike letters were significantly different ($p < 0.05$)

Conclusion: Previously, nicotianamine (NA) [and/or 2' deoxymugineic acid (DMA)] has been established as an enhance of Fe bioavailability in white wheat flour. This study investigated the effects of NA and biofortified wheat flour with NA and Fe *in ovo*. The study suggested that, both Fe/NA ($p = 0.008$) and Fe/EDTA ($p = 0.014$) improved serum Fe status and reduced *Ferroportin* expression compared to elemental Fe. The presence of Fe/NA also reduced *ACE*, *COX* and *AT1R* ($p < 0.05$) expression compared to the NI control. *Bifidobacterium* abundance increased ($p < 0.05$) and *Lactobillus*, *E. Coli* and *Clostridium* abundance decreased ($p < 0.05$) in the presence of Fe/NA compared to Fe/EDTA. All wheat extracts increased ($p < 0.05$) *Bifidobacterium* and *Lactobillus* abundance compared to NI and 18 MΩ H_2O controls. Both Fe/NA and HNWE1 elevated goblet cell numbers ($p < 0.05$) compared to Fe/EDTA and the LNWE, respectively. This study confirmed NA as a phytonutrient that promotes Fe absorption and positive gut health and reduces hypertension in *Gallus gallus*.

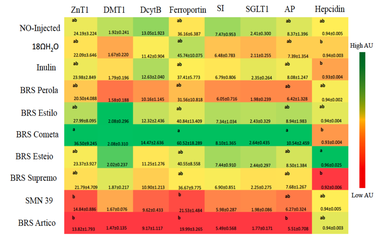
Carioca beans (*Phaseolus vulgaris*) prebiotics may improve the gut microbiota and iron related protein expression *in ovo* (*Gallus gallus*) (7)

Objective: This study aimed to assess the effect of prebiotics extracts from various beans varieties *in vivo* (*Gallus gallus*).

	Kaempferol 3-glucoside	Kaempferol	Catechin	Epicatechin	Procyanidin B1	Myricetin 3-glucoside	Quercetin 3-glucoside	Quercetin
Carioca beans								
BRS Perola (Standard)	17.3 ± 1.^{a}	-	26.1 ± 1.3^{a}	12.8 ± 1.7^{ab}	1.4 ± 0.2^{c}	-	0.2 ± 0.1^{d}	-
BRS Cometa (Biofortified)	16.2 ± 1.1^{a}	-	25.9 ± 4.6^{a}	11 ± 1.4^{b}	1.2 ± 0.2^{c}	-	-	-
Black beans								
BRS Esteio (Standard)	2.4 ± 0.4^{bc}	-	14.7 2.7^{b}	6.9 ± 0.6^{c}	0.7 ± 0.2^{de}	3.9 ± 0.3^{b}	2.0 ± 0.2^{b}	-
SMN 39	1.5 ± 0.2^{c}	-	13 ± 1.4^{b}	2.7 ± 0.7^{d}	3.0 ± 0.2^{a}	1 ± 0.1^{c}	0.9 ± 0.1^{c}	0.07 ± 0.01
White bean								
BRS Artico	0.8 ± 0.3^{c}	-	-	-		-	-	-

Table 1 . Concentration of polyphenol present in common bean flours (µM). Mean values within genes tested with unlike letters were significantly different ($p < 0.05$)

Figure 1 . Genera and species level bacterial populations from cecal contents in *Gallus gallus*. Mean values within genera tested with unlike letters were significantly different ($p < 0.05$)



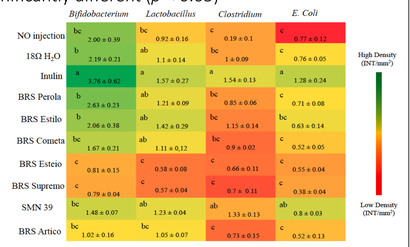


Figure 2 . Duodenum mRNA abundance of various genes in *Gallus gallus*. Mean values within genes tested with unlike letters were significantly different ($p < 0.05$)

Conclusion: This data suggests that the intra-amniotic administration of prebiotic extracts from carioca beans may improve Fe bioavailability by limiting potentially pathogenic bacterial population (*Costridium* and *E. coli*) and increasing of *Lactobacillus* and *Bifidobacterium*. The prebiotics extract from BRS Cometa upregulated gene expression of ZnT1, FPN and AP. Additionally, the carioca beans presented higher concentrations of polyphenols that can promote iron bioavailability compared to black beans. Thus, carioca beans can be an effective vehicle for mineral biofortification since they can improve the gut microbiota, hence improving the Fe bioavailability.

Conclusion

The intra-amniotic administration approach using *Gallus gallus* as an animal model is useful to determine the possible effects of plant origin nutrients and food ingredients on intestinal microbiota and BBM functionality.

References

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