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In ovo feeding of nutraceuticals and its role in adjusting the gastrointestinal tract, antioxidative properties, immunological response, and performance in poultry: An updated review

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Abstract: Nutraceuticals are food ingredients that provide extra health advantages that expand above their nutritional value. Nutraceuticals comprise amino acids, antioxidants, minerals, vitamins, probiotics, prebiotics, synbiotics, enzymes, organic acids, fatty acids, medicinal plants, etc. Recently, nutraceuticals have acquired much attention in poultry industry by reason of their potential effects on establishing the normal physiological status, supporting the immune system, and disease prevention, which consequently enhances productivity. The technique of *in ovo* feeding (IOF) of nutraceuticals holds several promises in poultry industry. The vital benefits of IOF of nutraceuticals are enhancement of intestinal development, establishment of a balanced microbial population in the gut, support of intestinal physiology and health. Interestingly, IOF of nutraceuticals participates in improving the antioxidative properties and energizing the immune system, which thereby translated into greater performance and bigger resistance to diseases, especially in early life challenges. The current review attempts to throw more light on the fresh results associated with the profits of IOF of nutraceuticals on intestinal histomorphology, intestinal microbiota, antioxidative properties, immune responsiveness, hatchability, chick quality, and growth performance in poultry.

Keywords: natural substance; intestinal histomorphology; immunity; growth

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The nutraceutical is a term that came from "nutrition" and "pharmaceutics". Nutraceuticals are food ingredients that provide extra health advantages that expand above their nutritional value. Recently, nutraceuticals have acquired much attention in poultry industry by reason of their potential impacts on establishing normal physiological status, supporting the immune system, and disease prevention, which consequently translated into enhancing productivity. Nutraceuticals include amino acids, antioxidants, minerals, vitamins, probiotics, prebiotics, synbiotics, enzymes, organic acids, fatty acids, medicinal plants, etc. (Alagawany et al. 2020). After banning the antibiotics as growth promoters, the world is looking forward to the natural alternatives of antibiotics because of the increasing demand for antibiotic-free and organic poultry products. Amino acids, vitamins, minerals, and enzymes are already used as dietary supplements to maintain the normal physiological status, support the immune response and enhance poultry productivity under thermo-neutral and heat stress conditions (Ebeid 2009, 2012; Saleh et al. 2019, 2020; Abdel-Moneim et al. 2021; Khalifah et al. 2022). Probiotics, prebiotics, synbiotics, and organic acids might be promising alternatives to antibiotic growth promoters in poultry production because of their capability to maintain balanced microbial populations in the gastrointestinal tract (GIT), establish intestinal integrity, and support the immune response in poultry (Wei et al. 2022; Ebeid and Al-Homidan 2022). Also, phytogenic feed additives, such as herbs, medicinal plants, seeds, and their extracts, play a fundamental role in enhancing feed utilization and improving the antioxidative status in poultry (Saleh et al. 2018; Al-Homidan et al. 2020; Fathi et al. 2020).

The technique of *in ovo* feeding (IOF) of nutraceuticals has attracted more attention because it holds several promises in enhancing the ability of newly hatched chicks to face challenges after hatching (Figure 1). The IOF of nutraceuticals improved intestinal morphometry and modulated a healthy intestinal microbial balance (Asa et al. 2022; Gonzales et al. 2022; Shehata et al. 2022). Also, IOF of nutraceuticals participates in improving the antioxidative properties and stimulating the immune response, leading to high growth performance (Yang et al. 2021; Dang et al. 2022; Mousstaaid et al. 2022) (Table 1). The current review attempts to throw more light on the fresh results associated with the profits

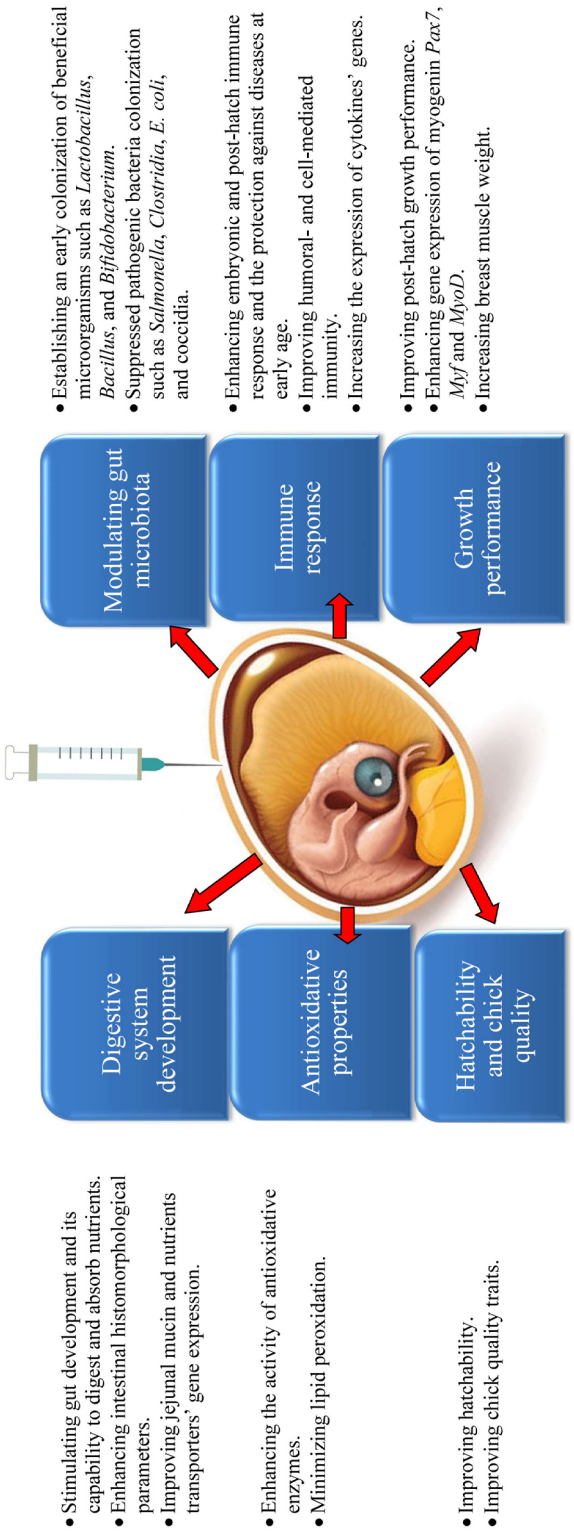

Figure 1. Main impacts of *in ovo* feeding of nutraceuticals on physiological and immunological aspects and performance in poultry

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Table 1. Summary of the impacts of *in ovo* feeding of nutraceuticals on gastrointestinal tract, antioxidative status, immune response, and performance in poultry

Bird	Nutraceuticals	Time of *in ovo* feeding	Main impacts	References
Broiler chickens	cysteine + lysine	day 17.5	• Improved hatchability and chick quality traits (navel area, scores of the eyes, appearance, activity, remaining membrane, legs, and the remaining yolk). • Improved post-hatch growth performance. • Improved intestinal morphology, including increased duodenal villus height, villus surface area and villus height/crypt depth ratio. • Increased serum superoxide dismutase and decreased serum malondialdehyde concentrations.	Ajayi et al. 2022
Geese	L-glutamine + L-leucine	day 23	• Increased breast muscle %, breast muscle glycogen content and diameter of myofibres at hatching. • Increased jejunal glutathione and glutathione peroxidase concentrations. • Elevated relative weight of small intestine. • Improved jejunal histomorphology (jejunal villus height, villus width, and villus surface area). • Enhanced jejunal digestive enzymes (maltase, sucrase, Na^+/K^+-ATPase, and alkaline phosphatase). • Upregulated jejunal nutrients transporter gene expression (jejunal sodium/glucose cotransporter protein-1 and jejunal glucose transporter-2).	Dang et al. 2022
Broiler chickens	*Bacillus subtilis* + raffinose	day 12.5	• Enhanced body weight, feed intake, and feed conversion ratio. • Increased ileal villus height, villus surface area and muscular thickness. • Increased caecal communities of *B. subtilis* and lactic acid bacteria. • Reduced caecal *E. coli*, *Enterococcus* spp., total coliforms, and total bacterial count. • Increased caecal concentrations of acetic, butyric, pentanoic, propionic, isovaleric, and isobutyric acid. • Increased ileal mRNA expression of interleukin-2 and toll-like receptor-4.	Shehata et al. 2022
Broiler chickens	L-ascorbic acid	day 18	• No negative effects on hatchability, hatching weight and quality of newborn chicks. • Reduced embryonic mortality. • Increased serum concentration of L-ascorbic acid and superoxide dismutase. • Lowered serum malondialdehyde concentration.	Mousstaaid et al. 2022
Broiler chickens	*Bacillus subtilis*	day 18.5	• No negative effect on hatching performance. • No negative effect on growth performance and mortality. • Enhanced villus height, villus width, and total mucosa thickness in jejunum and ileum. • Increased caecal concentrations of short-chain fatty acids.	Oladokun et al. 2021
Broiler chickens	*Nigella sativa* extract	day 17.5	• No negative effects on hatchability, hatching weight and quality of newborn chicks. • Enhanced body weight and feed conversion ratio. • Increased plasma superoxide dismutase. • Decreased plasma malondialdehyde concentrations.	Oke et al. 2021
Japanese quails	*Spirulina platensis*	day 15	• Increased hatchability and weight of newly hatched quail chicks. • Increased liver catalase activity and gene expression of liver glutathione peroxidase and reduced liver malondialdehyde concentration. • Increased liver interferon-γ gene expression. • Decreased gene expression of heat shock protein-70 in liver.	Hajati et al. 2021

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Table 1 to be continued

Bird	Nutraceuticals	Time of *in ovo* feeding	Main impacts	References
Broiler chickens	selenium nanoparticles	day 18	• Increased hatchability. • Increased body weight and body weight gain. • Increased serum glutathione peroxidase, superoxide dismutase and catalase. • Decreased serum malondialdehyde concentration.	El-Deep et al. 2020
Broiler chickens	zinc nanoparticles	day 17	• Increased plasma activities of glutathione peroxidase and superoxide dismutase. • Decreased plasma malondialdehyde concentration. • Decreased plasma concentrations of corticosterone, cortisol, cholesterol, triglyceride and glucose.	Shokraneh et al. 2020
Pigeons	L-lysine	day 13	• No significant effect on hatchability. • Improved body weight and body weight gain of squabs at 14 days of age. • Increased relative brain weight and organ index of heart, legs, and gizzard of squabs at 14 days of age. • Improved villus height, crypt depth, and villus surface area of jejunum.	Zhu et al. 2019
Broiler chickens	L-methionine + L-cysteine	day 17.5	• Increased hatchability and chick weight. • Increased serum concentration of glutathione peroxidase and total antioxidant capacity. • Increased villus area. • Increased serum heat shock protein-90.	Elwan et al. 2019

of IOF of nutraceuticals on intestinal histomorphology, intestinal microbiota, antioxidative properties, immune responsiveness, hatchability, chick quality, and growth performance in poultry.

Features of *in ovo* technology

Based on the published reports, the routes of IOF include amniotic fluid, allantoic membrane, air cell, yolk sac, and the embryo. Uni and Ferket (2004) suggested that the ideal time for IOF of nutrients into the amniotic fluid is the late phase of incubation, when the embryo consumes the amniotic fluid and the supplemental substances ingested directly with the amniotic fluid. The amniotic route is the optimal site in late stages of embryonic development and yolk sac in early stages of embryonic development. Application of IOF to late-stage embryos is safe coupled with no negative effects on hatchability but sometimes improved hatchability and chick quality (Nazem et al. 2017; Subramaniyan et al. 2019; Ajayi et al. 2022). Also, the site of IOF depends on the breed and embryonic development. Broiler eggs are usually injected into amniotic fluid or amniotic cavity on 17–18 days of incubation (Ajayi et al. 2022; Shehata et al. 2022), while pigeon eggs are injected into the amniotic cavity on day 13 of incubation (Zhu et al. 2019). Goose eggs are injected into the amnion on 24 days of incubation (Dang et al. 2022) and Japanese quail eggs are injected into the air sac on 15 days of incubation (Hajati et al. 2021). Under commercial conditions, IOF is carried out during egg transfer from setter to hatcher machine (on 17–18 days of incubation) to provide the high requirements for nutrients, vitamins, and minerals needed for rapid embryonic development. The IOF of prebiotics and synbiotics might be injected into the air cell on 12.5 days of incubation (Shehata et al. 2022), while probiotics might be injected into amniotic fluid or amniotic cavity on 17–18 days of incubation (Oladokun et al. 2021). This might be involved in establishing beneficial bacteria in chicken GIT.

Impact of *in ovo* feeding on digestive system development and intestinal histomorphology

Under normal circumstances, the GIT of day-old chicks has inadequate capacity to metabolize

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and utilize a diet rich in protein and carbohydrates (Uni and Ferket 2004). So, the prime objective of IOF is to accelerate the early GIT development and stimulate its capability to digest and absorb nutrients in the newly hatched chicks, and consequently supporting their fast-growth genes. Several reports confirmed that IOF of carbohydrates (Berrocoso et al. 2017; Slawinska et al. 2020; Asa et al. 2022), amino acids (Nazem et al. 2019; Gonzales et al. 2022; Reicher et al. 2022), and probiotics (Abdel-Moneim et al. 2020a; El-Moneim et al. 2020) improved the intestinal histomorphology. Earlier studies (Tako et al. 2004; Smirnov et al. 2006) proved that IOF of carbohydrates (sucrose, maltose, and dextrin) with or without β-hydroxy-β-methylbutyrate (a leucine metabolite) on day 17 or day 17.5 of embryonic development enhanced embryonic GIT development in form of improving the activities of digestive enzymes (sucrase-isomaltase and aminopeptidase) and enhancing the intestinal histomorphological parameters (e.g., higher villi, deeper crypts, higher villus/crypt depth ratio, and larger goblet cells). Furthermore, since goblet cells produce mucin, which inhibits dangerous infections from adhering to the epithelial surface, they are utilized as a sign of intestinal health (Ebeid et al. 2021a). Smirnov et al. (2006) indicated that IOF of carbohydrates activated the goblet cell proliferation and acidic mucin production. Similarly, the delivery of galactooligosaccharides via the *in ovo* route enhanced the intestinal health of chicks challenged with heat stress throughout the growing period (Slawinska et al. 2020). Asa et al. (2022) noted that IOF of 0.5 ml of carbohydrates and antioxidant mixture [maltose (25 mg/ml), sucrose (25 mg/ml), dextrin (100 mg/ml), insulin (1 IU/ml), vitamin E (12 mg/ml), Se (120 µg/ml), coenzyme Q10 (2 mg/ml), vitamin C (3 mg/ml), chromium picolinate (2 µg/ml), and dihydrostreptomycin (1 mg/ml)] improved the intestinal development in form of increasing the jejunum length and jejunum length/small intestine total length ratio. Moreover, they indicated that IOF of carbohydrates and antioxidant mixture elevated the villus length and decreased the crypt depth in the jejunum of newly hatched chicks. Also, Nazem et al. (2019) elucidated that IOF of dextrose (10.0 and 20.0%) and amino acid mixture (10.0%) into the yolk sac on day 14 of embryonic development accelerated the embryonic intestinal development via enhancing the villus length, villus width, and villus surface area in all small intestine segments. Dang et al. (2022) indicated that IOF of disaccharides (25 g/l maltose and 25 g/l sucrose) and/or methionine (5 g/l) elevated the relative weight of small intestine, enhanced jejunal digestive enzymes (maltase, sucrase, alkaline phosphatase, and Na^+/K^+-ATPase), upregulated the gene expression of jejunal nutrient transporters (jejunal sodium/glucose cotransporter protein-1 and jejunal glucose transporter-2), and jejunal histomorphology (villus height, width, and surface area) on 1, 7, and 28 days of age in geese.

Several recent studies postulated that IOF of amino acids enhanced GIT development and health (Gao et al. 2018a, b; Elwan et al. 2019; Reicher et al. 2022). Gonzales et al. (2022) revealed that IOF of a combination of sulphur amino acids (5.90 mg of L-methionine and 3.40 mg of L-cysteine) plus 0.15 mg of folic acid increased the ileal crypt depth in neonatal chicks. Also, Ajayi et al. (2022) demonstrated that IOF of a blend of 3.4 mg cysteine + 2 mg lysine on day 17.5 of incubation improved intestinal morphology, including elevated duodenal villus height, villus surface area, and villus height/crypt depth ratio. These results confirmed the previous studies, which showed that IOF of lysine enhanced the intestinal histomorphology in pigeons (Zhu et al. 2019). Reicher et al. (2022) confirmed that IOF of leucine and glutamine on day 17 of incubation increased the intestinal cell count by 33% and 40%, respectively, in comparison with the control. Importantly, they explained that supplementation of glutamine and leucine via the *in ovo* route accelerated the development and maturity of small intestine throughout the pre-hatch phase by upregulating the proliferation and differentiation of multipotent cells. Moreover, Gao et al. (2018a) elucidated that IOF of 1% L-arginine on day 17.5 of embryonic development enhanced the development of digestive organs (proventriculus, gizzard, and pancreas), improved the duodenal morphology (increased villus height, increased villus height/crypt depth ratio, and reduced crypt depth) and elevated the mucosal enzyme activities (maltase, sucrase, alkaline phosphatase, and nitric oxide synthase) on day 7 of age in broilers. At the level of gene expression, Gao et al. (2018b) elucidated that IOF of 1% L-arginine on day 17.5 of embryonic development was involved in regulating the intestinal barrier functions of post-hatch broilers via elevating the count of proliferating cell

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nuclear antigen-positive cells of villus, and gene expression of claudin-1, mucin-2, zonula occludens-1 and -2 in the jejunal mucosa of 21-day-old broilers. Bakyaraj et al. (2012) postulated that IOF of amino acids (serine, isoleucine and arginine) and trace elements (iron, iodine and zinc) improved mRNA expression of mucin.

Regarding natural antioxidants, Araujo et al. (2019) noted that IOF of vitamin E (27.5, 38.5, 49.5, and 60.4 IU) on day 17.5 of incubation increased small intestine % and duodenal villus height. These results agree with Rajkumar et al. (2015), who explained that IOF of vitamin E and glutamine helps in protecting the intestinal mucosa enterocytes, which consequently are involved in increasing cell proliferation and villus growth (Murakami et al. 2007). Furthermore, IOF of organic or inorganic Se on day 18 of incubation increased villus surface area and villus length and width in the duodenum and ileum (Krisnan et al. 2021).

Several studies indicated that the IOF of probiotics, prebiotics and/or synbiotics had a positive effect on GIT development (El-Moneim et al. 2020; Stadnicka et al. 2020; Boguslawska-Tryk et al. 2021; Shehata et al. 2022). Castaneda et al. (2021) concluded that IOF of *Enterococcus faecium*-based probiotic (1.4×10^7 CFU) on day 18 of incubation resulted in the heavier and longer jejunum, ileum, and caecum. Moreover, Boguslawska-Tryk et al. (2021) proved that IOF of probiotics, prebiotics and synbiotics enhanced villus height, villus width, and villus area in jejunum and ileum, which indicated a positive effect on the absorption surface area. Also, Shehata et al. (2022) documented that IOF of *Bacillus subtilis* (4×10^5 and 4×10^6 CFU), raffinose (2 and 3 mg), or their combinations increased ileal villus height, villus surface area and muscular thickness. Oladokun et al. (2021) confirmed that IOF of *B. subtilis* (10×10^6 CFU) on day 18.5 of incubation had a positive effect on intestinal morphometric characteristics, including villus height (18–23% higher), villus width (8% wider), and total mucosa thickness (28% greater) in jejunum and ileum giving a good indicator of the gut health and function. El-Moneim et al. (2020) observed that IOF of different kinds of probiotics (*Bifidobacterium bifidum* and *Bifidobacterium longum*) enhanced ileal histomorphology, including higher villus height and villus height/crypt depth ratio and reduced the crypt depth. Additionally, IOF of raffinose (1.5–4.5 mg/egg) enhanced ileum histomorphology and immunity, improving the gut health of newly hatched chicks (Berrocoso et al. 2017). Yang et al. (2021) indicated that the IOF of *Astragalus* polysaccharide (1, 2, and 4 mg) increased villus height and villus height/crypt depth ratio, while it reduced the crypt depth in intestinal segments at 7 and 21 days of age of broiler chickens. Interestingly, numerous studies explained that IOF of probiotics, prebiotics and/or synbiotics modulated the expression of intestinal function-related genes (mucin-2 and vascular endothelial growth factor) and nutrient transport-related genes (EAAT-3 and SGLT-1) in the ileum (Majidi-Mosleh et al. 2017; Pacifici et al. 2017; Pender et al. 2017; Shehata et al. 2022). Collectively, it is noteworthy to note that the IOF of nutraceuticals might be involved in improving GIT microarchitecture (villus-crypt units and goblet cells), enhancing the activities of digestive enzymes and consequently contributing to an improvement in nutrient absorption and utilization.

Impact of *in ovo* feeding of nutraceuticals on modulating gut microbiota

Gut microbiota plays an essential role in the GIT function and health. Thus, modulating GIT microbiota has been suggested as an effective strategy for boosting the host intestinal health, nutrient digestion, immunity, and productivity (Fathi et al. 2017, 2018; Ebeid et al. 2019). Unlike mammals, chicks hatch with a sterile GIT, and consequently, any contact with pathogenic bacteria poses a risk of infection and disease. The earlier colonization of GIT by useful bacteria supports birds in facing the environmental and pathogenic stressors (Abdel-Moneim et al. 2020b; El-Moneim et al. 2020). *In ovo* technology is a useful tool to establish the earlier colonization of beneficial microorganisms in the embryonic GIT. There is an extensive evidence illustrating that IOF of probiotics (Majidi-Mosleh et al. 2017; Pender et al. 2017; Castaneda et al. 2021), prebiotics (Berrocoso et al. 2017; Pacifici et al. 2017; Tavaniello et al. 2018; Stadnicka et al. 2020), and synbiotics (Boguslawska-Tryk et al. 2021; Shehata et al. 2022) helps to maintain a healthy microbial balance by strengthening the useful microorganisms like *Lactobacillus*, *Bacillus,* and *Bifidobacterium* and inhabiting the pathogenic bacteria such as *Salmonella*, *E. coli*, and *Clostridium*

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in GIT. Castaneda et al. (2021) detected that IOF of *B. subtilis* (10^6 CFU/50 µl/egg) on day 18 of incubation reduced pathogenic bacteria, including total aerobic bacterial counts and total coliforms in ileum and caecum on different days of the growth period of broilers. Also, Abdel-Moneim et al. (2020a) demonstrated that IOF of *Bifidobacteria* decreased total coliforms and total bacterial counts and enhanced *Bifidobacteria* and lactic acid bacterial counts in ileum. It was confirmed that IOF of probiotics decreased *Salmonella* colonization in caeca (de Oliveira et al. 2014; Teague et al. 2017). Teague et al. (2017) reported that IOF of commercial probiotic FloraMax®-B11 (lactic acid bacteria) reduced *Salmonella enteritidis* infection in broilers. These results agree with de Oliveira et al. (2014), who postulated that IOF of *E. faecium* reduced *S. enteritidis* challenges. This reduction of pathogenic bacteria might be attributed to the formation of short-chain fatty acids and antimicrobial peptides and bacteriocins, which produced beneficial bacteria (Majidi-Mosleh et al. 2017; Ebeid et al. 2021a).

The IOF of prebiotics had positive health profits in the host. Tavaniello et al. (2018) elucidated that IOF of raffinose motivated the early development of useful bacteria (*Lactobacilli* and *Bifidobacteria*) and suppressed pathogenic bacteria colonization in GIT. Similarly, Pacifici et al. (2017) and Stadnicka et al. (2020) illustrated that IOF of raffinose improved the gut health by decreasing the intestinal populations of *Clostridia*, *E. coli*, and coccidia counts and elevating the populations of *Bifidobacteria* and *Lactobacilli*. Likewise, Zhang et al. (2020) documented that IOF of chitooligosaccharide (5 mg) and chlorella polysaccharide (5 mg) on day 12.5 of incubation modulated caecal microbial community. The polysaccharide-utilizing bacteria such as *Lactobacillus johnsonii*, *L. crispatus*, *L. salivarius*, *Bacteroides coprocola*, *B. coprophilus*, and *B. salanitronis* were higher than those in control groups, while opportunistic pathogens such as *C. perfringens*, *Campylobacter jejuni*, *Fusobacterium mortiferum*, *Corynebacterium efficiens*, *Collinsella stercoris*, unclassified *Klebsiella*, *Shigella sonnei*, and *Shigella boydii* were lower than in the control.

Shehata et al. (2022) documented that IOF of synbiotics (*B. subtilis* plus raffinose) increased caecal communities of *B. subtilis* and lactic acid bacteria, and reduced *E. coli*, *Enterococcus* spp., total coliforms, and total bacterial count. These findings are in correspondence with Dunislawska et al. (2017), who reported that delivery of synbiotics (*L. salivarius* plus galactooligosaccharides and *L. plantarum* plus raffinose) via the *in ovo* route enhanced the populations of *Lactobacillus* spp. and *Enterococcus* spp. in ileum. Importantly, Shehata et al. (2022) documented that IOF of probiotics, prebiotics and/or synbiotics increased caecal contents of acetic, propionic, butyric, pentanoic, isovaleric, and isobutyric acids. These findings are in accordance with Boguslawska-Tryk et al. (2021). Also, Oladokun et al. (2021) reported that IOF of *B. subtilis* increased caecal concentrations of short-chain fatty acids in broilers. Also, raffinose plays a crucial role in accelerating *Lactobacillus* and *Bifidobacteria* fermentation and producing short-chain fatty acids that suppress the growth and proliferation of pathogens (Berrocoso et al. 2017). These organic acids lowered the intestinal pH and consequently played vital roles in suppressing GIT pathogens, supporting structural integrity, enhancing the barrier function of gut epithelia, physiological function, intestinal immunity and health (Ebeid and Al-Homidan 2022). Therefore, the earlier establishment of beneficial bacteria in chicken GIT can block pathogen colonization, enhancing GIT development and health, which finally translated into higher performance.

Impact of *in ovo* feeding of nutraceuticals on antioxidative status

Avian embryos are more sensitive to oxidative stress because of their extremely quick development, high metabolic rates, and high polyunsaturated fatty acid content in tissues. Therefore, activating the antioxidant defence system will protect the embryo against reactive oxygen species (ROS) (Deeming and Pike 2013). Surai et al. (2016) documented that the antioxidant system of developing embryo consists of antioxidative enzymes (glutathione peroxidase GSH-Px, superoxide dismutase SOD, and catalase CAT), water-soluble antioxidants (ascorbic acid, glutathione, taurine, carnitine, etc.), fat-soluble antioxidants (vitamin E, carotenoids, coenzyme Q) and antioxidant minerals (Se, Mn, Zn, etc.). The IOF of natural antioxidants enhances the activity of antioxidative enzymes, which play a fundamental role in scavenging and detoxification of ROS and also

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decreasing the malondialdehyde (MDA) concentration as an index of lipid peroxidation (Asa et al. 2022; Mousstaaid et al. 2022). Araujo et al. (2019) demonstrated that IOF of vitamin E (27.5, 38.5, 49.5, and 60.4 IU) on day 17.5 of incubation enhanced hepatic CAT activity of new-born chicks. Kalantar et al. (2019) noted that IOF of coenzyme Q10 (0.1 and 0.2 ml) on day 18 of incubation increased serum activities of CAT and SOD at 1–21 and 22–42 days of age in broilers. Moreover, IOF of a mixture of carbohydrates and antioxidants increased concentrations of CAT, SOD and total antioxidant capacity and decreased MDA concentration in the liver of neonatal chicks (Asa et al. 2022). Likewise, Oke et al. (2021) indicated that IOF of *Nigella sativa* extract (6 mg) increased plasma SOD and decreased plasma MDA concentrations of newly hatched chicks thermally challenged during incubation and also at marketing age (56 days). Also, Hajati et al. (2021) indicated that IOF of *Spirulina platensis* (2.5 or 3.5 mg) increased liver CAT activity and gene expression of hepatic GSH-Px and reduced liver MDA concentration in quail hatchlings.

It is widely known that IOF of L-ascorbic acid (vitamin C) at different levels enhanced the antioxidative properties and minimized lipid peroxidation (El-Senousey et al. 2018; Zhang et al. 2019; Mousstaaid et al. 2022). El-Senousey et al. (2018) reported that IOF of ascorbic acid (3 mg/egg) on day 18 of incubation elevated plasma GSH-Px and total antioxidant capacity concentrations and decreased MDA plasma concentration in post-hatched chicks. Mousstaaid et al. (2022) documented that IOF of 12 or 25 mg of L-ascorbic acid on day 18 of embryonic development increased serum concentrations of L-ascorbic acid and SOD and lowered serum MDA concentration. Similarly, Zhang et al. (2019) indicated that IOF of L-ascorbic acid (12 mg) increased plasma SOD and decreased MDA concentrations in 42-day-old broilers.

Numerous studies proved that IOF of antioxidant minerals, including Se (El-Deep et al. 2020; Krisnan et al. 2021), Mn (Geng et al. 2022), and Zn (Shokraneh et al. 2020) improved the antioxidative status and reduced lipid peroxidation. El-Deep et al. (2020) demonstrated that IOF of 10, 20, or 30 µg of nano-Se on day 18 of embryonic development enhanced serum concentrations of total antioxidant capacity, GSH-Px, SOD, and CAT. These results are in correspondence with Ibrahim et al. (2020), who showed that IOF of 10 ppb of nano-Se on day 18 of incubation increased serum concentrations of glutathione reductase and reduced MDA concentration at 35 days of broiler age. Also, Krisnan et al. (2021) indicated that IOF of 0.15 ppm of organic or inorganic Se on 18 day of incubation elevated serum GSH-Px activity at 18 days of chicken age. Moreover, Geng et al. (2022) noted that delivery of Mn (6.25, 12.5, 25.0, or 50.0 µg) via the *in ovo* route elevated SOD mRNA expression in the embryonic heart and might alleviate oxidative stress. Furthermore, Shokraneh et al. (2020) showed that IOF of 500 µg nano-Zn on day 17 of embryonic development increased serum activities of GSH-Px and SOD.

It was reported that amino acids have a greater antioxidant capacity via keeping a balance between producing and removing free radicals. Elnesr et al. (2019) revealed that IOF of sulphur amino acids (5.90 mg of L-methionine + 3.40 mg of L-cysteine) on day 17.5 of incubation enhanced the activities of GSH-Px, SOD, and CAT as well as it increased GSH-Px gene expression in serum, liver, jejunum, cardiac muscle, and pectoral muscle. These results are in correspondence with those of Elwan et al. (2019), who indicated that IOF of 5.90 mg L-methionine + 3.40 mg L-cysteine on day 17.5 of embryonic development increased the concentrations of total antioxidant capacity and glutathione in serum, liver, heart, kidney, pectoral muscle, and small intestine of newly hatched chicks subjected to hyperthermia during incubation. Recently, Dang et al. (2022) proved that IOF of methionine (5 g/l) and/or disaccharide (25 g/l maltose and 25 g/l sucrose) increased jejunal GSH and GSH-Px concentrations at seven and 28 days of age in geese. Han et al. (2022) postulated that IOF of L-leucine (69 µmol) increased serum GSH-Px and decreased serum MDA concentrations of broilers at 39 days of age. Also, Ajayi et al. (2022) demonstrated that IOF of a mixture of 3.4 mg cysteine + 2 mg lysine on day 17.5 of incubation increased serum SOD and decreased serum MDA concentrations of day-old chicks. Additionally, Lu et al. (2022) proved that IOF of 1% L-arginine on day 18 of incubation increased total antioxidant capacity and reduced MDA content of breast muscle at 21 days of age. It could be summarized that IOF of nutraceuticals might alleviate oxidative stress and improve the antioxidative status of developing embryos and post-hatch chicks.

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Impact of *in ovo* feeding of nutraceuticals on immune response

Modern broilers have become more sensitive to infectious diseases due to their high growth rate. Therefore, much attention was paid to improving the immune system and activating the immune response in the early growth stages. The IOF of nutraceuticals, immune stimulants, and bioactive components might be a promising tool to enhance the post-hatch immune response in poultry and grant protection against diseases (Salary et al. 2014; Subramaniyan et al. 2019; Yang et al. 2021; Shehata et al. 2022). It is well documented that IOF of natural antioxidants can enhance embryonic and post-hatch immunity and development at the early age. Bakyaraj et al. (2012) indicated that IOF of vitamin mixture (200 IU vitamin A, 20 IU vitamin D_3, 1 IU vitamin E, 72 µg thiamine, 144 µg riboflavin, 140 µg pyridoxine, 400 µg pantothenic acid, 140 µg niacin, and 8 mg vitamin C) or trace element mixture (0.3 µg Se, 80 µg Zn, 160 µg Fe, 0.7 µg I, 16 µg Cu, and 120 mg Mn) improved humoral immune response (antibody titre against sheep red blood cells SRBCs) and cell-mediated immunity (foot web thickness) at 21 days of age in broiler chickens. Salary et al. (2014) reported that IOF of 33.0 IU vitamin E increased serum antibody titres of avian influenza, infectious bronchitis, and Newcastle disease as well as it elevated serum values of immunoglobulins (IgG, IgA, and IgM) at 21 days of age of broilers. Additionally, Kalantar et al. (2019) noted that IOF of coenzyme Q10 (0.1 and 0.2 ml) on day 18 of incubation increased the weight of lymphoid organs (bursa of Fabricius and spleen) and serum antibody titres against Newcastle disease, infectious bronchitis disease, and avian influenza at 28 and 42 days of age in broilers. Hajati et al. (2021) indicated that IOF of *Spirulina platensis* (1.5, 2.5 or 3.5 mg) increased liver interferon-γ (IFN-γ) gene expression. Moreover, Li et al. (2016) demonstrated that IOF of 100 and 150 µg folic acid elevated plasma lysozyme activity and plasma concentrations of IgG and IgM in broiler chickens. They also elucidated that IOF of 100 and 150 µg folic acid upregulated gene expression of interleukin-2 (IL-2) and IL-4 and downregulated IL-6 gene expression, which indicated an improvement in the correlation between immunity and epigenetic regulation of cytokine genes.

The IOF of trace elements has gained extra attention due to their multiple physiological roles, such as activating several vital enzymes, promoting immunity, and alleviating the oxidative stress. Krisnan et al. (2021) indicated that IOF of 0.15 ppm of organic or inorganic Se on day 18 of incubation improved the antibody titre against Newcastle disease virus and lymphocyte and heterophil percentages. These results are consistent with Ibrahim et al. (2020), who illustrated that IOF of 10 ppb of nano-Se on day 18 of incubation increased serum concentrations of IgM, IgG, and total immunoglobulins and antibody titre against Newcastle disease virus. Goel et al. (2016) noted that IOF of trace elements (500 µg Zn, 17.5 µg I, or 1.5 µg Se) on day 14 of incubation improved humoral and cell-mediated immunity via increasing the expression of cytokine genes (IL-2, IL-12, and TNF-α) in post-hatch broiler chickens.

Bakyaraj et al. (2012) indicated that IOF of amino acid mixture (10 mg methionine, 16 mg threonine, 25 mg arginine, 12.5 mg glycine, 12.5 mg serine, and 18 mg valine) improved the antibody titre against SRBCs and cell-mediated immunity at 21 days of age in broiler chickens. In another study, Toghyani et al. (2019) reported that IOF of arginine (35 mg) and/or threonine (25 mg) on day 14 of incubation increased the antibody titre against SRBCs significantly, while antibody titres against Newcastle disease and avian influenza were not increased significantly. Moreover, IOF of arginine significantly increased the relative weights of the bursa of Fabricius and spleen in comparison with the sham control at 11 days of age in broilers. Similarly, Gonzales et al. (2022) revealed that IOF with a combination of sulphur amino acids (5.90 mg of L-methionine and 3.40 mg of L-cysteine) plus 0.150 mg of folic acid increased the relative weights of thymus and spleen at 21 days of age. Moreover, it was demonstrated that IOF of 100 µg L-arginine (Subramaniyan et al. 2019) or 100 µg L-arginine conjugated with 1 000 µg silver nanoparticles (Subramaniyan et al. 2020) on day 14 of incubation increased the serum concentration of IgM in newly hatched chicks. In the same line, Kermanshahi et al. (2017) stated that IOF of 0.5 mg threonine on day 11 of embryogenesis upregulated IgA mRNA in the new-born chicks of Japanese quail.

Probiotics can stimulate both specific and non-specific immune responses by encouraging mac-

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rophages, boosting cytokine production, and increasing IgA, IgG, and IgM concentrations (Fathi et al. 2017; Ebeid et al. 2021a, b). The IOF of probiotics, prebiotics, and synbiotics is a promising tool for the earlier establishment of useful bacteria in GIT and consequently it stimulates the intestinal innate (nonspecific) immunity and adaptive (specific) immune response. Alizadeh et al. (2021) noted that IOF of 1×10^7 CFU *Lactobacilli* at embryonic day 18 improved the antibody titre against SRBCs and keyhole limpet haemocyanin, elevated the proportion of $CD4^+$ and $CD4^+CD25^+$ T cells in spleen and upregulated gene expression of interferon (IFN)-α, IFN-β, IL-8, IL-13, and IL-18 in spleen and gene expression of IFN-γ, IL-2, IL-6, IL-8, IL-12, and IL-18 in the bursa of Fabricius. Also, Pender et al. (2017) postulated that IOF of probiotics (*L. acidophilus*, *L. casei*, *E. faecium*, and *B. bifidum*) downregulated the expression of Toll-like receptors-2 and -4, inducible nitric oxide synthase, trefoil factor-2, mucin-2, INF-γ, and IL-4 and IL-13 in ileum and caecal tonsils, which participate in regulating the innate and adaptive immune responsiveness. Moreover, Yang et al. (2021) indicated that IOF of *Astragalus* polysaccharide (1 mg, 2 mg, and 4 mg) on day 18 of incubation enhanced the intestinal mucosal immunity in form of increasing the number of IgA^+ cells in mucosa and the secretory IgA concentrations in intestinal washings, which consequently support the first barrier against pathogen invasion. Moreover, levels of relative mRNA expression of IL-2, IL-4, IFN-γ, and Toll-like receptor-4 were elevated due to IOF of *Astragalus* polysaccharide. Furthermore, Shehata et al. (2022) documented that IOF of *B. subtilis* (4×10^5 and 4×10^6 CFU), raffinose (2 and 3 mg), or their combinations increased ileal mRNA expression of IL-2 and toll-like receptor-4. These results are consistent with those of Dunislawska et al. (2017), who documented that IOF of synbiotics (*L. salivarius* + galactooligosaccharides and *L. plantarum* + raffinose) resulted in a significant upregulation of IL6, IL18, IL1-β, IFN-γ, and IFN-β in spleen on day 21 of age and it also resulted in a significant downregulation of IL-12, IL-8, and IL-1β on day 42 and IFN-β in caecal tonsils on day 14 of age. Collectively and by taking into consideration the antioxidative properties of nutraceuticals, IOF of nutraceuticals can motivate the humoral and cell-mediated immunity via regulating the gene expression of cytokines.

Impact of *in ovo* feeding of nutraceuticals on hatchability and chick quality

Several studies elucidated that IOF of amino acids improved hatchability and chick quality (Nazem et al. 2017; Subramaniyan et al. 2019; Ajayi et al. 2022). Bakyaraj et al. (2012) indicated that IOF of amino acid mixture enhanced hatchability per fertile egg set, chick weight and chick weight/egg weight ratio. Another study by Nazem et al. (2017) also noted that IOF of methionine elevated the weight at hatch, which might be attributed to the enhanced antioxidant status in embryos. Ajayi et al. (2022) demonstrated that IOF of a mixture of 3.4 mg cysteine + 2 mg lysine on day 17.5 of incubation improved hatchability and chick quality traits (scores of eyes, legs, navel area, appearance, activity, remaining membrane, and remaining yolk). Similarly, Subramaniyan et al. (2019) demonstrated that IOF of L-arginine (100 µg) on day 14 of incubation improved hatchability, survival rate, and body weight of newly hatched chicks. These improvements might be attributed to the fact that embryos may use the *in ovo* delivered amino acids to improve the energy status and build muscle protein, which finally translated into improving hatchability, chick quality, and survival rate.

Several recent publications documented the positive effect of IOF of antioxidants, vitamins, and minerals on hatchability and chick quality characteristics (El-Deep et al. 2020; Hajati et al. 2021; Geng et al. 2022). Asa et al. (2022) observed that IOF of a mixture of antioxidants and carbohydrates improved hatching %, chick weight, chick quality characteristics, appearance and eye condition, and activity of new-born chicks. Araujo et al. (2019) noted that IOF of 60.4 IU vitamin E improved hatchability %, shortened the hatch window, and enhanced chick physical quality parameters (newborn chick quality score, body weight, length, and chick weight/egg weight ratio). El-Senousey et al. (2018) proved that IOF of ascorbic acid (3 mg/egg) increased chick weight and chick weight/egg weight ratio. Kalantar et al. (2019) noted that IOF of coenzyme Q10 (0.1 and 0.2 ml) on day 18 of incubation increased the hatching rate and body weight of newly hatched chicks and this improvement might be associated with the enhancement of the antioxidant status, which grants protection against the oxidative stress. Bakyaraj et al. (2012) indicated that IOF of vitamin mixture or trace element mixture enhanced hatchability per fertile egg set, chick weight and

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chick weight/egg weight ratio. In addition, Hajati et al. (2021) indicated that IOF of *Spirulina platensis* (1.5, 2.5 or 3.5 mg) increased hatchability and weight of newly hatched quail chicks. Li et al. (2016) demonstrated that IOF of 100 and 150 µg folic acid enhanced hatchability. El-Deep et al. (2020) illustrated that IOF of 10, 20, or 30 µg of nano-Se on day 18 of incubation enhanced hatchability. Furthermore, Geng et al. (2022) noted that IOF of 25.0 µg Mn enhanced hatchability, but embryonic mortality and hatched chick weight were not significantly affected. It might be mentioned that the IOF of exogenous antioxidants, vitamins, and minerals reduces free radical production and enhances lipid utilization for getting the energy needed for hatching.

On the other side, several studies reported that IOF of probiotics (Pender et al. 2017; Teague et al. 2017; Alizadeh et al. 2021; Castaneda et al. 2021), prebiotics (Stadnicka et al. 2020; Yang et al. 2021) and synbiotics (Dunislawska et al. 2017) had no significant effect on hatchability and chick quality. Studies reported that IOF of *Lactobacilli* (Alizadeh et al. 2021), *E. faecium* and *B. subtilis* (de Oliveira et al. 2014), *Pediococcus acidilactici*, *E. faecium*, and *B. subtilis* (Majidi-Mosleh et al. 2017) did not affect hatchability, embryonic mortality (early, mid, and late death), pipped, contaminated, and culled embryos and chick weight. Also, IOF of raffinose (Stadnicka et al. 2020) and *Astragalus* polysaccharide (Yang et al. 2021) had no significant influence on hatchability. Likewise, Dunislawska et al. (2017) reported that IOF of synbiotics (*L. salivarius* + galactooligosaccharides and *L. plantarum* + raffinose) had no negative effect on hatchability. Likewise, several studies elucidated that IOF of nutraceuticals, including Se (Hassan 2018; Krisnan et al. 2021), vitamin E (Salary et al. 2014; Rajkumar et al. 2015), L-ascorbic (Mousstaaid et al. 2022), *Nigella sativa* extract (Oke et al. 2021), and L-methionine plus 3.40 mg L-cysteine (Elwan et al. 2019) had no negative effects on hatchability, hatching weight and quality of new-born chicks. It might be reported that if the IOF of nutraceuticals does not improve the hatching traits, it will not harm them.

Impact of *in ovo* feeding of nutraceuticals on growth performance

Increasing live body weight (BW) and body weight gain (BWG) that are directly related to the increase in average daily feed intake (ADFI) and the improvement in feed conversion ratio (FCR) serve as indicators of improvements in growth performance. The majority of studies elucidated that IOF of nutraceuticals enhanced growth performance. Several publications documented that IOF of a mixture of carbohydrates and antioxidants (Asa et al. 2022), Se (Hassan 2018; El-Deep et al. 2020; Krisnan et al. 2021), coenzyme Q10 (Kalantar et al. 2019), *Nigella sativa* extract (Oke et al. 2021), and folic acid (Li et al. 2016) improved growth performance measurements. Additionally, a series of recent studies have indicated that IOF of amino acids enhanced growth performance (Yu et al. 2018; Toghyani et al. 2019; Ajayi et al. 2022; Dang et al. 2022; Gonzales et al. 2022). Ajayi et al. (2022) demonstrated that IOF of a mixture of 3.4 mg cysteine + 2 mg lysine on day 17.5 of embryogenesis enhanced post-hatch growth performance. Similarly, Toghyani et al. (2019) postulated that IOF of L-arginine (35 mg) and/or threonine (25 mg) on day 14 of incubation increased BW, BWG, and ADFI. Zhu et al. (2019) documented that IOF of L-lysine (2.11 mg) increased BW and BWG of squabs at 14 days of age. Also, Dang et al. (2022) proved that IOF of methionine (5 g/l) and/or disaccharides (25 g/l maltose and 25 g/l sucrose) increased breast muscle %, breast muscle glycogen content and diameter of myofibres at hatching in geese. They also illustrated that IOF of methionine upregulated the expression of muscle fibre development genes, including myogenic factor-5 and myostatin, at 1, 7, and 28 days of age in geese. The IOF of amino acids or peptides increases the pectoral muscle mass via the expression of muscle development genes. It was documented that the expression of myogenin and MyoD was significantly upregulated by IOF of L-arginine (100 µg) on day 14 of incubation (Subramaniyan et al. 2019). Likewise, Subramaniyan et al. (2020) confirmed that IOF of L-arginine conjugated with silver nanoparticles (100 µg with 1 000 µg, respectively) on day 14 of incubation upregulated the protein expression of muscle development markers (myogenin and *myoD*). Also, Gonzales et al. (2022) detected that IOF of 0.150 mg folic acid increased the expression of the *Pax7* and *Myf* genes involved in satellite cell proliferation in the pectoralis muscle of hatched chicks. Moreover, Yu et al. (2018) postulated that IOF of 1.0% L-arginine enhanced energy reserves of liver, pectoral muscle, and serum

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thyroid hormone concentrations. Furthermore, Gao et al. (2018a) demonstrated that IOF of 1% arginine improved digestive enzyme activities, which finally translated into enhancing the post-hatch growth performance of broilers. It might be reported that IOF of nutraceuticals upregulated the protein expression of muscle development and also it increased muscle glycogen (energy) stores, which promote myofibre maturation, leading to enhancement of the muscle size.

The IOF of probiotics, prebiotics, and synbiotics positively impacted on GIT development and its physiological function. Several authors have recognized that IOF of probiotics (El-Moneim et al. 2020; Castaneda et al. 2021), prebiotics (Tavaniello et al. 2018; Yang et al. 2021), and synbiotics (Shehata et al. 2022) enhanced post-hatch growth performance traits. These supplements may play a part in improving the health condition of GIT by boosting the colonization of beneficial bacteria, reducing the colonization of pathogenic bacteria, promoting intestinal morphology, increasing activities of digestive enzymes, and enhancing feed digestibility and utilization (Abdel-Moneim et al. 2020a; El-Moneim et al. 2020; Ebeid et al. 2021a, b).

On the other side, IOF of nutraceuticals does not always grant a positive impact on growth performance in poultry. It was observed that IOF of *B. subtilis* (Oladokun et al. 2021), *B. subtilis*, *E. faecium,* and *Pediococcus acidilactici* (Majidi-Mosleh et al. 2017), raffinose (Stadnicka et al. 2020), or *L. salivarius* + galactooligosaccharides and *L. plantarum* + raffinose (Dunislawska et al. 2017) had no beneficial effect on live BW, BWG, ADFI, FCR, and mortality throughout the growing period. Also, Han et al. (2022) postulated that IOF of L-leucine had no significant influence on BWG and relative weights of liver, heart and bursa of Fabricius; however, it improved the relative weight of spleen in broilers at 39 days of age. Similarly, Geng et al. (2022) noted that IOF of 25.0 μg Mn did not affect BWG, ADFI, FCR, mortality, dressing %, breast muscle %, thigh muscle %, abdominal fat %, pH and colour of breast and thigh meats.

Conclusion

Based on the recent findings, it could be concluded that IOF of nutraceuticals might be a promising tool to enhance poultry productivity under thermo-neutral and heat stress conditions. The vital benefits of IOF of nutraceuticals are enhancement of intestinal development, establishment of the healthy intestinal microbial balance, and support of the intestinal function and health. Interestingly, IOF of nutraceuticals participates in enhancing the antioxidative properties and activating the immune system, which thereby translated into high performance and bigger resistance to diseases. Indeed, obtaining such advantages is essential in the commercial poultry industry, especially for antibiotic-free poultry production.

Conflict of interest

The authors declare no conflict of interest.

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