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Research Article

Modification of Growth and Gene expression in Coloured Broilers by Early Post-Hatch Micronutrients Supplementation

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Abstract

The effect of early post-hatch additional dietary supplementation of Biotin and lysine (B+L), Folacin and lysine (F+L), Biotin (B) or Folacin (F) alone was studied in coloured broilers. Birds under dietary treatments received either control diet or supplemental feed with either additional 0.15 mg biotin or 0.55 mg folacin and with 1100 mg lysine/kg or without lysine for up to 14 days of age depending upon the treatments. The relative hepatic mRNA expression of Chicken Growth hormone (cGH), Insulin like growth factors 1 and 2 (IGF-1 and 2) and Myostatin (MSTN) gene were quantified through real-time PCR at 7- and 14-days post-hatch. The results showed that body weight gain during 0-14 and 0-42 days improved ($P < 0.001$) with folacin and lysine supplementation, while FCR was better during 0-42 days. At 7 and 14 days, there was increase in mRNA expression of cGH and IGF-1 genes in all treatments with maximum expression in folacin + lysine group, while inverse relationship existed in Myostatin expression. However, IGF-2 mRNA expression did not corroborate with body weight gain in any treatments. In conclusion, dietary biotin, folacin and lysine supplementation to chicks during 0-14 days of age enhances early growth by modifying growth related genes expression.

Keywords: Early Post-Hatch Feeding; Micronutrients; Growth; Gene Expression; Broilers

Abbreviations

cGH: Chicken Growth Hormone; IGF 1 and 2: Insulin Growth Factor 1 And 2; MSTN: Myostatin; ME: Metabolisable Energy; CP: Crude Protein; B. W. G: Body Weight Gain; FCR: Feed Conversion Ratio

Introduction

Growth is a complex structural and functional phenomenon that depends on species, genetic background, metabolic status, protein complement of the muscle and environmental factors [1] Among these, diet is the most important environmental factor that exerts profound impact on genome and phenotypic expression during fetal and post-natal development. The availability of critical nutrients during the early post-hatch period not only improves chickens' lifetime performance but also reduces the marketing age and production cost of broilers. The increased metabolic needs of birds at young age require specific micro-nutrients, especially some B-complex vitamins like biotin, folacin etc. These micro-nutrients, besides their well-known roles as substrates and cofactors in carboxylation reaction and single carbon metabolism, have pivotal role in DNA synthesis, gene expression, cell viability, growth and differentiation of tissues and maturation of immune system. Hence nutrient deficiencies during the first few days after hatch will impair chick development and compromise immune function at the beginning of their lives which reflects in the post-natal growth and meat production [2].

Nutrient supply regulates overall body growth directly or indirectly through its influence on regulatory factors. Several studies conducted on growth factors revealed that circulating Insulin like

growth factors (IGF-1 and IGF-2) as well as hepatic IGF-1 mRNA expression was regulated by nutritional state [3,4]. It has been proved that energy deficiency or protein depletion, as well as specific micro-nutrient deficiency can inhibit the production of IGF-I [5,6]. Further nutritional regulation of IGF-I expression appears to be tissue specific and the variation of mRNA level was more in liver, muscle and kidney than in other tissues, such as stomach and heart. Similarly, Myostatin (MSTN) a negative regulator of muscle growth was found sensitive to the nutrient supply as the mRNA levels of MSTN remained low in Sartorius muscle during fasting period [7].

Moreover, the role of critical dietary micronutrients on the production performances and their mechanism in the expression profile of growth-related genes is still obscure in broiler chickens. Hence, keeping in view the above facts, an endeavor was made to assess the effect of the micro-nutrients (biotin, folacin and lysine) on growth parameters and expression profile of different growth-related genes viz., Growth hormone (GH), Insulin like growth factors (IGF 1 and 2) and Mysostatin (MSTN) gene in broiler chickens during early post-hatch development.

Materials and Methods

Germplasm and experimental procedures

Day-old coloured broiler chicks (n = 200) were randomly distributed among five treatment groups (including control) with 5 replicates of 8 birds in each (mean chick weight ± 2.0 g). All the chicks were reared in battery brooders under standard nutritional and management practices up to 42 days. The control group (T1) received corn-soy basal starter diet (l2.6MJ ME/kg, 207g/kg CP) so compounded as in table 1 for 0-21 d and finisher diet (12.6 MJ ME/kg, 193g/kg CP) from 22-42 d of age. While the day-old chicks of the rest 4 treatments were also given ad libidum access to the same basal diet of control but along with additional supplementation of D-biotin (S.D. Fine Chem Ltd., India), Folacin (98% pure, SRL Pvt Ltd., India) and lysine (L- lysine hydrochloride CDH Laboratory Reagents, India) above National Research Council, 1994 recommendations [8] such that. T2 received additional 0.15mg D-biotin and 1100mg lysine (B+L), T3 received additional 0.55mg folacin and 1100mg lysine (F+L), T4 received additional 0.15mg D-biotin only (B) and T5 received only additional 0.55mg Folacin (F) per kg basal diet. The experimental diet was fed only up to 14 days and thereafter standard basal starter diet up to 21 days and finisher diet from 22-42 days, that is, birds of all groups received similar diet from 15-42 days of age. The body weight changes and feed intake were recorded weekly up to 42 days of age.

Gross composition (g/kg)	Starter ration	Finisher ration
Maize	621	676
SBM	335	293
Vegetable oil	11	02
Limestone powder	12	13
Di-calcium phosphate	145	10
Trace mineral premix*	0.1	0.1
Vitamin premix**	0.1	0.1
Sodium chloride	03	03
Methionine	0.21	0.11
B-Complex***	0.02	0.02
Choline chloride	0. 05	0.05
Toxin binder	0.5	0.05
Chemical composition (calculated on the basis of gross composition)		
ME (MJ/kg)	12.6	12.6
CP (g/kg)	207	193
ME: CP	144.6	155.3
Lysine	1.10	1.00
Methionine	0.5	0.38
Threonine	0.8	0.74

Table 1: Ingredient composition of broiler basal diets fed during starting (0-21 days) and finishing (22-42 days) phases of experimental birds.

*Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn-30 and Cu- 4 mg/kg diet.

**The vitamin premix supplied vitamin A- 8250 IU, vitamin D_3-1200 ICU; vitamin K- 1mg; vitamin E- 40 IU.

***Vitamin B_1- 2mg, vitamin B_2- 4mg, vitamin B_{12}-10mcg; niacin-60mg; pantothenic acid-10mg; choline-500mg/kg diet.

RNA isolation and reverse transcription

Total RNA was isolated from liver tissue of three chicks from each treatment at 7 and 14 days of age using Trizol reagent (Invitrogen, USA). The purity and quantity were assessed by measuring the optical density of each sample at 260 versus 280 nm. Any possible traces of genomic DNA were removed by treating 5µg of each RNA sample with 5U of RNase-free DNase (Biogene, CA, USA) at 37 ºC for 1 hour. The DNase was subsequently inactivated by incubation at 65 ºC for 10 min. Each DNase treated total RNA sample (5µg) was reverse transcribed using the RevertAid First strand

cDNA synthesis kit (MBI Fermentas, Hanover, MD, USA) according to the manufacturer's instructions. The resultant cDNA was stored at -20°C until used. Negative controls were performed using all components except reverse transcriptase.

Estimation of IGF-I, IGF-II, cGH and MSTN by real time PCR

After reverse transcription of the target mRNA into cDNA, it was quantified by real-time PCR using Syber Green mastermix in Stratagene MX3000. The gene-specific primer pairs shown in table 2 were designed to amplify the genes of interest. 28S rRNA gene was used as endogenous control. For each individual sample (n=3 per group) genes viz. IGF-1, IGF-2, cGH, MSTN, cDNA were amplified in triplicates. The amplification was carried out in 25µl volume containing 1X QuantiTect SYBR Green PCR master mix (SYBR Green 1 dye, HotStart Taq DNA polymerase and dNTPs in optimized buffer components; QIAGEN GmBH, Germany), a 0.2µM concentration of each gene-specific primer and 1µl of cDNA template. PCR cycling conditions were: initial denaturation of 95°C for 10 minutes, followed by 40 cycles of denaturation 95°C for 30 seconds; annealing for 30 seconds and extension 72°C for 45 seconds. For each gene of interest, negative and positive controls were included. Negative controls were samples in which cDNA was not added.

A melting curve was performed for each sample after completion of amplification and analyzed in comparison to negative and positive controls, to determine the specificity of PCR reaction. Relative hepatic gene expression was calculated as ΔCt (cycle threshold) of the experimental sample by normalizing Ct value of target gene using housekeeping gene (28s rRNA) i.e., subtracting the Ct average of the experimental samples and Ct value of the experimental blank 28s rRNA (house-keeping gene).

Gene	Primer	Annealing temperature (°C)	Amplicon size (base pair)	Reference/ Accession No.
cGH	F-CAC CAC AGC TAG AGA CCC ACA TC R-CCC ACC GGC TCA AAC TGC	58	199	AF 289468/D10484
IGF-1	F- TGT ACT GTG CTC CAA TAA AGC R- CTG TTT CCT GTG TTC CCT CTA CTT G	58	197	M32791
IGF-2	F- GAA ATA ACA GGA GGA TCA ACC GTG R- TTC TTC TGCC ACA CGT TGT ACT TG	55	201	NM30342
MSTN	F- GCT TTTG ATG AGA CTG GAC GAG R- AGC GGG TAG CGA CAA CATC	59	173	[7]
28s rRNA	F- GGC GAA GCC AGA GGA AAC T R- GAC GAC CGA TTT GCA CGT C	55	62	X59733

Table 2: Primer sequences of growth-related genes used for PCR amplification.

ΔCt = ΔCt (target gene)- ΔCt (house-keeping gene)

The ΔCt values were then normalized using control group to obtain ΔΔ Ct

ΔΔ Ct = ΔCt (Experimental sample) - ΔCt (Control sample)

The relative quantity of target gene was calculated using the formula stated by [9]

Relative quantity = $2^{-\Delta\Delta Ct}$

Statistical analysis

Data emanated from different treatments were analyzed for statistical significance by following standard methods [10]. Results were considered as significant at the level of 95%

($P < 0.05$) for comparison.

Results

Body weight gain and feed conversion ratio

The results for body weight gain, feed intake, feed conversion ratio (FCR) presented in table 3. revealed that there was significant improvement ($P < 0.001$) in body weight gain in all supplemented groups than control during 0-2 weeks as well as during 0-6-week period. Maximum body weight gain was recorded in T3 group, followed by T2, T5 and T4 group with about 145g, 114g, 100g and 95g

respectively of higher weight gain than control during 0-6-week period. However, the feed intake was almost similar among the groups throughout the growth period. There was no significance difference in FCR during 0-2 weeks but significant difference ($P < 0.05$) was observed in FCR between supplemented groups and control during 0-6-week period, with better FCR observed in T3 group and apparently poor FCR in T1 group.

Parameters		Dietary treatments (N = 200)						
		T1 (Ctl) (n = 40)	T2(B+L) (n = 40)	T3(F+L) (n = 40)	T4(B) (n = 40)	T5(F) n = 40)	SEm	Prob
B.W.G (g/bird)	0-2 weeks	230^{S}	248^{Q}	253^{P}	241^{RS}	243^{R}	0.63	P<0.001
	0-6 weeks	1466^{T}	1580^{Q}	1611^{P}	1561^{S}	1566^{RS}	2.70	P<0.001
Feed intake (g/ bird)	0-2 weeks	410.4	412.0	410.4	411.3	409.8	0.118	NS
	0-6 weeks	3429	3431	3430	3431	3428	0.187	NS
FCR	0-2 weeks	1.61	1.59	1.58	1.59	1.58	0.002	NS
	0-6 weeks	2.24^{P}	2.12^{R}	2.11^{S}	2.11^{S}	2.13^{Q}	0.006	P < 0.05

Table 3: Performance of birds subjected to early post hatch micronutrients supplementation during 0-14 days.

Values bearing different superscripts in a row vary significantly; NS = Non-Significant ($P > 0.05$)

Ctl: Control; B+L: Biotin and lysine supplementation; F+L: Folacin and lysine supplementation; B: only Biotin supplementation; F: only Folacin supplementation.

mRNA expression profile of growth-related genes

The relative hepatic expressions of the genes considered in this study at 7 and 14 days of age are presented in Figure 1 and 2 respectively. It may be noted from the current study that IGF-1 and cGH expression has increased with age and the relative levels of IGF-1 expression was parallel to cGH expression. The expression was consistently higher in all supplemented groups than control at both periods of gene analysis. The T3 group showed significantly higher ($P < 0.05$) mRNA expression of IGF-1 and cGH as compared to their counterparts at 7 and 14 days of age. Similarly, T2 group also showed significantly higher ($P < 0.05$) expression of IGF-1 and cGH genes as compared to all other groups except T3 during post hatch growth. The relative hepatic IGF-2 expression decreased during 7-14 days period in all treatment groups and the expression was high in T4 and T5 at 7 days of age and T5 and T1 at 14 days of age. On the other hand, relative hepatic MSTN gene expression was low in all supplemented groups than control and least expression was observed in T3 group at 7 and 14 days of age.

Discussion

Body weight gain and feed conversion ratio

The significant improvement in body weight gain and FCR with no considerable changes in feed intake during 0-6-week period in all supplemented groups especially in T3 group suggests that early post-hatch dietary supplementation of biotin or folacin along with or without lysine can influence utilization of nutrients and neo-natal growth of chicks. Nevertheless, these observations corroborate with the findings of [11-13], wherein sole dietary supplementation of folacin or lysine increased the bird's performance. However, in our study enhanced performance was observed in chicks supplemented with both folacin and lysine than with folacin alone. This may be due to the associative action of folacin in absorption of lysine and other amino acids (except isoleucine) [14]. Lysine, a limiting amino acid, associated with protein accretion and growth was found to stimulate intestinal development and better absorption and utilization of nutrients [15] and thereby improve weight gain, feed efficiency and breast meat yield with its increasing dietary levels in broilers [16]. The supplementation of optimal levels of dietary lysine in the feed was found to increase systemic or localized production of growth factors such as IGF-1 in broiler chicken [17], which in turn may stimulate DNA synthesis in chicken muscle satellite cells and thereby accelerate skeletal muscle development [18].

Folates have central role in gene transcription and nucleic acid synthesis that are absolutely necessary for proper functioning of cell division, organogenesis and growth and moreover its requirement is high during early growth phase and in animals with greater growth or production rates [19] Further early post hatch feeding of folacin, may stimulate intestinal growth and increase absorption of nutrients and thereby aid in greater nutrient assimilation and better growth.

Figure 1: mRNA expression of cGH, IGF-1, IGF-2 and MSTN at 7th day post hatch in treatment groups.
Ctl: Control; B+L: Biotin and lysine supplementation; F+L: Folacin and lysine supplementation; B: only Biotin supplementation; F: only Folacin supplementation
Different superscripts showing significant differences at ($P < 0.05$) level.

Figure 2: mRNA expression of cGH, IGF-1, IGF-2 and MSTN at 7th day post hatch in treatment groups.
Ctl: Control; B+L: Biotin and lysine supplementation; F+L: Folacin and lysine supplementation; B: only Biotin supplementation; F: only Folacin supplementation.
Different superscripts showing significant differences at ($P < 0.05$) level.

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mRNA expression profile of growth-related genes

The relative mRNA expression profile of different growth-related genes namely IGF-1, IGF-2, cGH, and MSTN in liver at 7 and 14 days of age revealed that expression of IGF-1 increased with increasing age of the birds. The present observation of higher IGF-1 expression is in relation with the findings of [20], who reported that hepatic IGF-1 mRNA expression increased at 1, 3, 5 and 7 weeks of post hatch period. Further, the present data showed that the hepatic expression of IGF-1 and cGH was enhanced with feeding of micronutrients as compared to control. The T3 group showed significantly higher ($P < 0.05$) mRNA expression of IGF-1 and cGH as compared to their counterparts at 7 and 14 days of age. Correspondingly, T2 group also showed significantly higher ($P < 0.05$) expression of these two genes as compared to all other groups

except T3 during post hatch growth. The relative levels of IGF-1 expression were parallel to cGH expression and showed consistent higher expression in all supplemented groups particularly in T3 group than control and other groups at both periods of gene analysis. This parallel increase in the expression of these growth promoting (cGH and IGF-1) genes is coherent with the findings of [21]. Further, the higher body weight of birds during 0-2 weeks period in all supplemented treatments with maximum expression in T3 chicks suggests that dietary supplementation of folacin and lysine during first 2 weeks accelerate expression of cGH and IGF-1 genes and promotes growth. The current result reaffirms the findings of previous studies by [17] that optimum levels of dietary lysine result in systemic or localized production of growth factors like IGF-1 that stimulate satellite cell mitotic activity and growth.

The relative hepatic IGF-2 expression decreased during 7-14 days period in all treatment groups and this is in agreement with the findings of [22] wherein, IGF-2 was expressed at comparable levels in brain and liver tissue during embryonic development, except for transient increases in liver just prior to hatching (days 24 and 26) and at 3 weeks post-hatch in turkey. In this study relative IGF-2 expression was high in T4 and T5 group on 7 days of age and T5 and T1 on 14 days of age The groups which showed higher relative IGF-2 expression had relatively lesser body weight gain during 0-2-week period and showed little consistent influence on body weight gain. These observations corroborate with previous findings that IGF-2 concentrations were unrelated to post hatch growth rate and has no clear growth-promoting effect in chickens but rather may favour lipid deposition in birds [23,24].

On the other hand, relative hepatic MSTN gene expression was low in all supplemented groups than control with least expression in T3 group at 7 and 14 days of age. Present observation of least expression of MSTN in liver of individuals of T3 group is in agreement with the earlier reported findings [25]. The inverse relationship observed in MSTN gene expression with respect to body weight and other growth promoting genes at 7 and 14 days of age in all the treatments suggests that MSTN serve as a negative regulator of muscle growth in birds as reported by previous studies [26,27] MSTN (GDF-8) inhibits the activation, proliferation, and differentiation of myogenic satellite cells [28] and exerts pleiotropic effects on broiler performance with significant associations to growth and meat quality [29] and thus facilitate the anabolic effects of cGH [27].

Conclusion

The corresponding changes in mRNA expression of growth-related genes to that of bird's performance in various treatments suggests the existence of nutrient-gene interaction. Hence dietary supplementation of the micronutrients namely, biotin, folacin and lysine during the first two weeks of early post hatch period may act as genetic modulators of growth by altering the expression of growth-related genes namely cGH, IGFs and MSTN and important determinants of satellite cell mitotic activity for ultimate skeletal muscle growth in coloured broilers.

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Conflict of Interest

All authors declare that they have no conflict of interest.

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