

NICOTINAMIDE RIBOSIDE *IN OVO* FEEDING EFFECTS ON HIGH-YIELD BROILER *PECTORALIS MAJOR* MUSCLE MYOGENESIS, MUSCLE WEIGHT, AND MUSCLE FIBER MORPHOMETRICS

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I. INTRODUCTION

Nicotinamide riboside (NR) is a vitamin B3 analog which numerous studies demonstrated increased mice muscle satellite cell number and proliferation rate in mice [1]. Previous studies reported *in ovo* feeding NR to commercial- and high-yield broilers affected *Pectoralis major* muscle (PMM) weight and fibre density [2,3]; however, effects of these changes post hatch remain unexplored. Additionally, previous literature showed muscle enhancement may cause muscle myopathies in broilers [4]. The objective of this study was to evaluate effects of *in ovo* NR feeding on PMM whole and fibre morphometrics and histological myopathy classification at hatch and after 48 days of growth.

II. MATERIALS AND METHODS

Fertilized Cobb 700 eggs (N = 3,260) were placed on incubation trays, trays were weighed and assigned to one of four treatments: eggs injected with 0 (NR0), 250 (NR250), 500 (NR500), or 1,000 (NR1000) mM NR at incubation day 10. At hatching, chicks were sexed, female chicks (N = 30/treatment) were euthanized for whole PMM morphometrics, and males were randomly allocated to 32 pens (N = 18/pen) which contained *ad libitum* access to feed and water. At day 48 post-hatch, 3 birds per pen were randomly selected, euthanized, and PMM were removed, weighed, cut in half, and blotted for muscle CSA measurement. The centre portion of the PMM cranial area was collected, cryopreserved, cryosectioned, and wheat germ agglutinin was used to identify muscle cell walls. In addition, PMM muscle was paraffin embedded, sectioned, and stained with Mason Trichrome and hematoxylin/eosin to classify fibres as Trichrome co-stained, degenerating, or normal. Data were analysed as a completely randomized design with bird as the experimental unit and Treatment as the fixed effect. Statistical significance was determined at $P < 0.05$ and trends or tendencies were determined at $0.05 > P > 0.10$.

III. RESULTS AND DISCUSSION

There were treatment effects for hatched-chick whole PMM weight and length ($P < 0.02$; Table 1). *Pectoralis major* weight of NR0 birds did not differ ($P = 0.23$) from NR250 birds, but weighed less than NR500 and NR1000 PMM ($P < 0.03$) which did not differ ($P = 0.26$). Birds from 250NR treatment did not differ ($P = 0.35$) in PMM weight compared to NR500 birds, but weighed less ($P = 0.04$) than NR1000 PMM. *Pectoralis major* length of NR0 birds did not differ from NR250 and NR500 birds ($P > 0.17$), but were less ($P = 0.02$) than NR1000 PMM. Birds from 250NR treatment tended to have less ($P = 0.06$) PMM length than NR500 birds, but were less ($P < 0.01$) than NR1000 PMM. Length of NR500 and NR1000 PMM did not differ ($P = 0.32$). There was no treatment effect ($P = 0.22$) for day-48 chicken whole-PMM CSA, but there were treatment effects for PMM weight and fibre number ($P < 0.05$). Birds injected with 0 mM NR had less PMM weight than all NR inject birds ($P < 0.02$),

who did not differ from each other ($P > 0.26$). Birds injected with 0 mM NR had larger muscle fibre CSA than 500NR and 1000NR ($P < 0.03$) and tended to have larger ($P = 0.07$) muscle fibre CSA than 250NR birds. All NR injected birds did not differ in muscle fibre CSA ($P > 0.49$). Birds injected with 0 mM NR had less ($P < 0.01$) muscle fibre number than 500NR and tended to have less muscle fibre number than 250NR and 1000NR birds ($P < 0.09$). All NR injected birds did not differ in muscle fibre number ($P > 0.18$). There were no treatment effects on day-48 PMM percentage of fibres labelled as Trichome co-stained, degenerating, or normal ($P > 0.22$).

Table 1 Effects of *in ovo* nicotinamide riboside feeding on hatched-chick and day-48 chicken *pectoralis major* whole muscle and fibre morphometrics¹.

Item	Nicotinamide riboside, mM				SEM	P-value
	0	250	500	1,000		
Hatched chicks						
Whole muscle weight, g	0.19 ^a	0.20 ^{a,c}	0.21 ^{b,c}	0.22 ^b	0.006	<0.01
Length, mm	21.8 ^a	21.5 ^{a,x}	22.5 ^{a,b,y}	23.1 ^b	0.41	0.02
Day-48 chickens						
Whole muscle weight, g	956 ^a	1,013 ^b	995 ^b	994 ^b	13	<0.01
Whole muscle CSA ² , cm ²	484	497	509	482	10	0.22
Fibres, # ×10 ⁶	8.11 ^{a,b}	9.80 ^{a,b,y}	10.72 ^b	9.53 ^{a,b,y}	0.656	0.03
Trichome co-stained ³ , %	8.0	8.2	9.1	11.3	1.24	0.22
Degenerating fibres, %	8.2	10.8	9.5	11.0	1.22	0.31
Normal fibres, %	83.8	81.0	81.4	77.8	2.21	0.27

¹Hatched chicks were female, while day-48 chickens were male. ²CSA; cross-sectional area. ³Fibers which appeared purple due to Trichome and H&E co-staining. ^{a,b}Means within a row with different letters differ ($P \leq 0.05$). ^{x,y}Means within a row with different letters tend to differ ($P \leq 0.10$).

IV. CONCLUSION

The advantages at hatch in PMM weight due to high-yield broiler *in ovo* NR feeding is maintained after 48 days of post-hatch growth. While day-48 PMM weight does not increase with increasing dose, PMM fibre number increased, and NR *in ovo* feeding had no effect on muscle fibre myopathy classification.

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