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Effect of Arginine Deficiency on Normal and Dystrophic Chickens[1] (38604)

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Inherited muscular dystrophy has been reported in New Hampshire chickens by Asmundson and Julian (1) and Asmundson *et al.* (2). Dystrophic chickens have difficulty in righting themselves when placed on their backs due principally to an abnormality in the pectoralis muscle. This muscle initially shows a hypertrophy and later an atrophy with a destruction of muscle fibers and their replacement by fat (Jordan *et al.* (3)).

The need for arginine in the diet of the chick has been established (4 and 5) and a deficiency results in poor growth and a muscular weakness. From the fact that a muscular weakness is noted in both the inherited muscular dystrophy and with a deficiency of arginine, we decided to investigate the effect of a deficiency of arginine on the development of dystrophy in both normal chicks and chicks with inherited dystrophy. Lysine is antagonistic to arginine (6–9) and the addition of lysine to an arginine deficient diet was used as a means of further aggravating this deficiency.

Materials and Methods. Two experiments were conducted in which normal New Hampshire chickens or genetically dystrophic chickens (line 307) were fed the experimental diets for 28 days. The basal diet was essentially similar to the casein diet described previously (9) and the amino acid supplements mentioned in Table I were added at the expense of glucose. In all cases except for chicks in Group 1, experiment 2, each group of normal chicks were divided into two and was housed with half of the dystrophic chicks which were fed the same diet to minimize environmental effects. The birds were raised in electrically heated batteries with raised wire floors and were provided feed and water *ad libitum*.

The dystrophic condition was evaluated by examining the birds twice a week by placing them on their backs and recording the number of times they could right themselves before becoming exhausted. Body weights were recorded twice each week and at the end of the experiment all of the chickens were killed and samples of the gastrocnemius and superficial pectoralis muscle taken for later analysis. The tissue samples were kept frozen until the creatine plus creatinine analysis was carried out according to the method of Rose *et al.* (10) which was adapted for autoanalyzer use. In experiment 1, the tissue was also analyzed for moisture and fat content by the method described by Jordan *et al.* (3). Statistical analyses were carried out by the *t* test.

Results. In experiment 1, body weight gains (Table I) were significantly reduced when the diet was deficient in arginine and further significantly reduced with the addition of L-lysine. Body weight gain was more severely restricted in the normal chicks deficient in arginine or those fed lysine than in the dystrophic strain. In experiment 2, one comparable group deficient in arginine (Group 4) showed a difference similar to those noted in experiment 1. Fairly similar growth responses were also noted in Groups 1–3 in which no glycine was added but in which a deficiency of arginine was produced. In all cases, growth was more severely restricted in the normal chicks with a deficiency of arginine than in the dystrophic chicks and the growth depression was aggravated by the addition of lysine to the arginine deficient diet.

The flip test scores for normal chicks showed no difference among dietary treatments in each experiment. In the dystrophic chicks, however, the muscular ability of the chicks was decidedly poorest in those groups receiving arginine and was markedly improved (from 3.4–7.7 and 12.1 in exp. 1 and from 1.0–6.7 and 6.4 in exp. 2) in those

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TABLE 1. BODY WEIGHT GAINS OF NORMAL AND DYSTROPHIC CHICKENS FED A CASEIN DIET WITH VARIOUS AMINO ACID SUPPLEMENTS FOR 28 DAYS.

	Supplement	Normal chicks		Dystrophic Chicks		Significance of difference
		Number	Body wt. gain, g.	Number	Body wt. gain, g.	
Exp. 1						*P*
1	Glycine, 0.6%	12/12[a]	45.3 ± 4.5	11/12	116.5 ± 9.6	<0.001
2	Glycine, 0.6% L-Lysine·HCl 1.25%	11/12	18.8*** ± 2.9[b]	12/12	41.5*** ± 2.9	<0.001
3	Glycine, 0.6% L-Arginine·HCl, 0.96%	12/12	314.5 ± 12.2	11/12	281.9 ± 11.9	ns
Exp. 2						
1	None	5/5	68.4 ± 7.6	5/5	215.4 ± 14.0	<0.001
2	L-Lysine·HCl, 1.25%	6/10	15.8*** ± 2.5[c]	8/10	74.1*** ± 9.7	<0.001
3	L-Arginine·HCl, 0.96%	10/10	372.4*** ± 24.6	8/10	389.0*** ± 13.5	ns
4	Glycine, 0.6%	8/10	39.8** ± 4.2	7/10	138.3*** ± 14.5	<0.001
5	Glycine, 0.6% L-Arginine·HCl, 0.96%	7/10	365.4*** ± 25.9	8/10	407.9*** ± 12.0	ns

[a] Number of survivors/original number.
[b] *** Indicates significantly different from Group 1 at $P < 0.001$.
[c] ** and *** Indicates significantly different from Group 1 at $P < 0.01$ and <0.001, respectively.

birds deficient in arginine or aggravated by the addition of lysine. The results were quite similar in both experiments.

The total creatinine present in the leg (gastrocnemius) muscle (Table I) was slightly higher in the group receiving arginine compared with the group deficient in arginine in experiment 1, although the difference was not statiscally significant in experiment 2. In dystrophic leg muscle there was a greater increase with the addition of arginine than in normal muscle. The most striking and consistent differences were noted in the pectoral muscle where the values for dystrophic chick muscle from groups fed an arginine deficient diet were lower than comparable groups of normal chicks fed the same diets. With arginine supplemented groups there was no difference between normal and dystrophic chicks in the total creatinine content of their muscle.

Since the gross composition of dystrophic muscle is so different from normal muscle, the total creatinine in experiment 1 was also expressed in terms of milligrams per gram of fat-free dry tissue (Table II). When expressed in this way the total creatinine content of the leg muscle and the pectoral muscle was significantly greater in the arginine supplemented than in the arginine deficient controls. In the leg muscle, the dystrophic birds had slightly greater total creatinine in the arginine deficient group and considerably greater amounts in the arginine supplemented group than in the normal birds. In the pectoral muscle, the arginine deficient dystrophic birds had significantly less total creatinine than their normal controls. These differences are relatively similar to those noted in the creatinine values expressed as milligrams per gram of fresh tissue.

Discussion. The reduced growth noted in an arginine deficiency is similar to that which was reported previously (5 and 9) and the antagonism with arginine by lysine had also been reported previously (6–9). The markedly smaller growth depression caused in dystrophic chicks with a deficiency of arginine has not been noted previously and appears to indicate that these chicks have a lower dietary requirement for arginine than normal chicks. Hutt and Nesheim (11) and Austic and Nesheim (12) previously have studied a strain of birds which has a low requirement for arginine.

While there was a marked improvement in the ability of chicks to right themselves when they were deficient in arginine, the possibility exists that this is a mechanical rather than physiological mechanism. Those chicks which were fed the diets deficient in arginine or in which the arginine deficiency was further aggravated with the addition of

TABLE II. TOTAL CREATININE COMPOSITION OF MUSCLE OF CHICKENS FED VARIOUS SUPPLEMENTS.

		Total creatinine in muscle (mg/g fresh tissue)					
		Leg Muscle			Pectoral		
		Normal	Dystrophic	P^a	Normal	Dystrophic	P
Exp. 1							
1	Glycine, 0.6%	3.15 ± 0.09	3.02 ± 0.09	ns	3.40 ± 0.13	2.31 ± 0.09	<0.001
2	Glycine, 0.6% L-Lysine·HCl, 1.25%	3.65 ± 0.28	3.00 ± 0.07	<0.05	4.12 ± 0.37	2.48 ± 0.11	<0.001
3	Glycine, 0.6% L-Arginine·HCl, 0.95%	3.42[b]* ± 0.07***	4.33 ± 0.20	<0.001	3.80* ± 0.09	3.65*** ± 0.16	ns
Exp. 2							
1	None	3.11 ± 0.29	3.15 ± 0.08	ns	3.77 ± 0.56	2.51 ± 0.20	ns
2	L-Lysine·HCl, 1.25%	2.58 ± 0.16	2.78 ± 0.15	ns	3.40 ± 0.13	2.37 ± 0.26	<0.01
3	L-Arginine·HCl, 0.96%	2.92 ± 0.20	4.15 ± 0.14	<0.001	3.46 ± 0.06	3.71 ± 0.18	ns
4	Glycine, 0.6%	2.72 ± 0.22	2.91 ± 0.16	ns	3.78 ± 0.28	2.33 ± 0.17	<0.001
5	Glycine, 0.6% L-Arginine·HCl, 0.96%	3.38 ± 0.24	4.04[c]*** ± 0.12	<0.05	3.40 ± 0.31	3.65*** ± 0.09	ns
Exp. 1		*Total creatinine (mg/g fat-free dry tissue)*					
1	Glycine, 0.6%	11.23 ± 0.28	12.64 ± 0.43	<0.05	10.68 ± 0.40	8.88 ± 0.62	<0.05
2	L-Lysine·HCl, 1.25%	13.67* ± 0.88	12.15 ± 0.45	ns	13.79* ± 1.35	10.08 ± 0.53	<0.05
3	Glycine, 0.6% L-Arginine·HCl, 0.96%	13.55*** ± 0.49	17.88*** ± 0.76	<0.001	13.59* ± 1.04	15.25*** ± 0.47	ns

[a] Level for significant differences between means of normal and dystrophic chicks.
[b] * and *** indicate means are significantly different from those of Group 1 at $P < 0.05$ and 0.001 levels.
[c] *** indicates means are significantly different from those of Group 4 at $P < 0.001$ level.

lysine were much smaller than the control chicks. This apparent increase in muscular ability may be related to the smaller size rather than improved physiological function.

The muscle creatinine data in most cases showed that there was a slight reduction in muscle creatine with a deficiency of arginine as had been reported previously (5 and 13). Perhaps the most significant observation is the fact that in the pectoral muscle, which is most severely affected by the hereditary dystrophic condition, the muscle creatine is considerably lower in the muscle of the dystrophic birds fed an arginine deficient diet than in normal birds given the same treatment. It would appear that the dystrophic birds have a lower dietary requirement for arginine, but, on the other hand, the ability to synthesize creatine from arginine is considerably reduced. There is, possibly, a greater diversion of the arginine which is available for functions related to growth and less is available for creatine synthesis. Ingwall *et al.* (14) have shown that in tissue culture studies creatine is involved in the control of muscle protein synthesis.

Summary. Normal and genetically dystrophic chickens were fed diets deficient in arginine or further aggravated by the addition of lysine. Growth of dystrophic chicks is depressed less by a deficiency of arginine than that of normal chicks. Muscular ability of dystrophic chicks was improved by feeding them an arginine deficient diet but this effect may be related to the size of the bird. The total creatinine content of the pectoral muscle of dystrophic chicks was reduced more with a deficiency of arginine than was that of normal chickens.

1. Asmundson, V. S., and Julian, L. M., J. Hered. **47,** 248 (1956).
2. Asmundson, V. S., Kratzer, F. H., and Julian, L. M., N. Y. Acad. Sci. Ann. **138,** 49 (1966).
3. Jordan, J. P., Kratzer, F. H., Johnson, A. M., and Freeman, R. L., Proc. Soc. Exp. Biol. Med. **101,** 449 (1959).
4. Klose, A. A., Stokstad, E. L. R., and Almquist, H. J., J. Biol. Chem. **123,** 691 (1938).
5. Almquist, H. J., Mecchi, E., and Kratzer, F. H., J. Biol. Chem. **141,** 365 (1941).
6. O'Dell, B. L., and Savage, J. E., J. Nutr. **90,** 364 (1966).
7. Lewis, D., Smith, G. H., and Payne, C. G., Brit. J. Nutr. **17,** 415 (1963).
8. Jones, J. D., J. Nutr. **84,** 313 (1964).
9. Kadirvel, R., Vohra, P., and Kratzer, F. H., J. Nutr. **104,** 1127 (1974).
10. Rose, W. C., Helmer, O. M., and Chanutin, A. J., Biol. Chem. **75,** 543 (1927).
11. Hutt, F. B., and Nesheim, M. C., Can. J. Genet. Cytol. **8,** 251 (1966).
12. Austic, R. E., and Nesheim, M. C., Poultry Sci. **51,** 1098 (1972).
13. Fisher, H., Salander, R. C., and Taylor, M. W., J. Nutr. **59,** 491 (1956).
14. Ingwall, J. S., Morales, M. F., and Stockdale, F. E., Proc. Nat. Acad. Sci. **69,** 2250 (1972).

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