



# Journal of Biological Sciences

ISSN 1727-3048

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## Study of Oral Creatine Monohydrate Supplementation on Growth Performance and Histopathological Assessment in Rats and Chickens

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**Abstract:** To clarify the effects of Creatine Monohydrate (CrM) supplementation on growth performance and to evaluate histopathological lesions in rats and broiler chickens. Two species of animals, chickens and rats were maintained on standard conditions. Each of species (thirty six rats and thirty six chickens), were separated into three equal groups of twelve animals. The control group receiving no CrM while the experimental groups received CrM added to the water supply to achieve a dose of 0.25 and 0.5 g kg<sup>-1</sup> day<sup>-1</sup>. The body weight and feed intake were measured and feed conversion rate was calculated. All animals were dissected after 15 days; Portions of liver, kidney, skeletal muscle, were separated and prepared for histopathological sections. There were not any significant variations in body weight gain and feed conversion rate between control and creatine-supplemented groups in rats. There were not also any change in feed conversion rate in chickens but body weight gain significantly ( $p < 0.05$ ) increased at day 15 of rearing in creatine-supplemented group (0.5 g kg<sup>-1</sup> day<sup>-1</sup>) compared to its control. Histopathological changes were not significant in two species. This study showed that creatine do not have any pathological effect on liver, kidney and muscle of rats and chickens and can improve body weight gain in rearing chickens.

**Key words:** Creatine monohydrate, rat, chicken, growth performance

### INTRODUCTION

Creatine is a guanidine compound produced endogenously by the liver and kidneys and is consumed in meat-containing diets (Terjung *et al.*, 2000). It is transported to skeletal muscle, heart, brain and several other tissues by a sodium-dependent transporter (Wyss and Kaddurah-Daouk, 2000). Endogenous synthesis of creatine plus dietary contribution, with half excreted as creatinine (Juhn and Tarnopolsky, 1998a, b). Creatine is an important compound in cellular energy buffering and in the shuttling of energy from the mitochondria to the cytosol. Creatine functions in maintaining cellular ATP homeostasis (Brosnan and Brosnan, 2007). Fatigue during maximal exercise of short duration is partially the result of phosphocreatine (PCr) depletion and inability of phosphocreatine hydrolysis to maintain a high ATP: ADP ratio (Greenhaff, 1997). Thus, nonendurance athletes routinely ingest creatine for a short-term loading period (Toler, 1997). During high-

intensity exercise, ATP hydrolysis is initially buffered by PCr via the Creatine Kinase (CK) reaction. Whereas PCr is available instantaneously for ATP regeneration, glycolysis is induced with a delay of a few seconds and stimulation of mitochondrial oxidative phosphorylation is delayed even further. On the other hand, the PCr stores in muscle are limited so that during high-intensity exercise, PCr is depleted within 10 sec. Therefore, if it were possible to increase the muscle stores of PCr and thereby to delay PCr depletion, this might favorably affect muscle performance. Studies have found that dietary supplementation with Creatine Monohydrate (CrM) can increase skeletal muscle (Parise *et al.*, 2001) and brain (Leuzzi *et al.*, 2000) total creatine and phosphocreatine concentrations, with an even greater degree of increase seen in organs with low baseline creatine content such as liver and kidney (Ipsiroglu *et al.*, 2001).

Creatine loading results in increased water retention (Juhn, 1999). Cellular hydration is an anabolic proliferative signal for protein synthesis (Persky and Brazeau, 2001).

Additionally, Creatine monohydrate increases bone mineral density (Antolic *et al.*, 2007). Thus, creatine supplementation could be expected to increase weight gain and percentage lean.

There has been some concern regarding the potential for CrM toxicity based on two anecdotal human case reports (Koshy *et al.*, 1999; Thorsteinsdottir *et al.*, 2006), one animal study in hypertensive rats (Edmunds *et al.*, 2001) and the fact that carcinogens can be formed if creatine and sugars are heated to high temperatures (Wyss and Kaddurah-Daouk, 2000). In humans, most of the studies that have examined the potential for toxicity have not found evidence of side effects when consumed at recommended doses (Kreider *et al.*, 2003; Mihic *et al.*, 2000; Schilling *et al.*, 2001). Several recent reviews have concluded that dietary CrM supplementation in humans appears to be relatively safe in the short term; however, they cautioned that the long-term side effects have not been evaluated systematically (Juhn and Tamopolsky, 1998b; Terjung *et al.*, 2000). Encouragingly, more recent studies in humans have not found evidence for CrM-associated toxicity based on blood analysis and side-effect questionnaires in older adults (Brose *et al.*, 2003), young athletes (Terjung *et al.*, 2000) and in patients with neurological diseases (Jan Groeneveld *et al.*, 2003; Walter *et al.*, 2000).

This study was conducted to examine the effects of supplementation of CrM on growth performance and to evaluate its related histopathological lesions in rats and broiler chickens.

## MATERIALS AND METHODS

### Animals

**Chickens:** The thirty five -day-old fast -growing broiler chickens (Ross 308) were reared for two weeks in floor pens (density 12 chickens m<sup>-2</sup>) on shaving saw dust litter. All chickens were kept under standard conditions (temperature, light) and provided *ad libitum* access to water and a standard ration.

**Rats:** Young Sprague-Dawley rats were used in this study. They were maintained on standard chow and tap water *ad libitum* and were housed under conditions of controlled temperature (25±1 °C) and light (07:00-19:00 h). The experimental protocol was designed in compliance with the Principles of Laboratory Animal Care under constitutional rules of Sharekord University.

Each of species (thirty six rats and thirty six chickens), were randomly separated into three equal

groups of twelve animals and housed in these groups. The control group receiving no CrM while the experimental groups received CrM (Merck Chemical Co., Bubendorf, FRG) added to the water supply to achieve a dose of 0.25 and 0.5 g kg<sup>-1</sup> day<sup>-1</sup>.

**Experiments:** Throughout the study, behavior of animals was noted and mortality was recorded daily. The body weight and feed intake were measured daily from each group and feed conversion rate was calculated.

All animals were euthanized using an overdose of Halothane after 15 days of maintenance and were immediately dissected; tissues were visualized and palpated for evidence of gross pathology. Portions of liver, kidney, skeletal muscle (Iliotibialis), were immersed in 10% phosphate-buffered formaldehyde (formalin). These samples were dehydrated in increasing concentrations of ethanol and xylene and embedded in paraffin and 5 µm thick sections were stained with hematoxylin and eosin and cover slipped. The tissue sections were reviewed blindly for histopathological changes.

**Statistical analysis:** The tissue histopathology was analyzed using the Chi-Square nonparametric statistical test (SPSS-14.0 package). For body weight, feed intake and feed conversion rate as mean±SEM, a one-way ANOVA was employed with Tukey's post hoc test (SPSS-14.0 package). A p-value <0.05 was taken to indicate statistical significance.

## RESULTS AND DISCUSSION

It was observed changes in the behavior of experimented rats after day 7. These changes were as hyperactivation and increased aggressiveness but it was not observed any change in behavior of chickens during rearing.

There were not any significant variations in body weight gain and feed conversion rate between control and creatine-supplemented groups in rats (Table 1). There were not also any change in feed conversion rate in chickens but body weight gain significantly (p<0.05) increased at day 15 of rearing in creatine-supplemented group (0.5 kg<sup>-1</sup> day<sup>-1</sup>) compared to its control (Table 2). The tissue sections showed nonsignificant histopathological changes such as congestion, hyaline cast, sinusoidal dilatation, proximal tubular cellulitis in the liver or kidney of both species (Fig. 1, 2). No change was observed in muscles.

Table 1: Comparison of mean body weight gain and feed conversion rate in different rat groups

Parameters	Days	n	Control	T <sub>1</sub> (0.25 g kg <sup>-1</sup> day <sup>-1</sup> )	T <sub>2</sub> (0.25 g kg <sup>-1</sup> day <sup>-1</sup> )
Body weight gain (g)	3	12	161±10	167±8	156±4
	6	12	167±10	173±8	161±4
	9	12	176±9	178±9	166±5
	12	12	184±10	187±8	168±4
	15	12	191±10	198±9	171±4
Feed conversion rate	3	12	0.02±0.001	0.01±0.002	0.02±0.003
	6	12	0.01±0.002	0.01±0.001	0.02±0.001
	9	12	0.01±0.002	0.01±0.002	0.02±0.001
	12	12	0.01±0.001	0.01±0.001	0.01±0.001
	15	12	0.01±0.001	0.01±0.001	0.01±0.001

Table 2: Comparison of mean body weight gain and feed conversion rate in different broiler groups

Parameters	Days	n	Control	T <sub>1</sub> (0.25 g kg <sup>-1</sup> day <sup>-1</sup> )	T <sub>2</sub> (0.25 g kg <sup>-1</sup> day <sup>-1</sup> )
Body weight gain (g)	3	12	1260±41	1217±65	1254±60
	6	12	1550±39	1457±50	1545±54
	9	12	1822±46	1861±91	1769±55
	12	12	2223±90	2113±66	2197±78
	15	12	2349±59	2487±55	2512±55*
Feed conversion rate	3	12	0.09±0.008	0.10±0.007	0.09±0.004
	6	12	0.10±0.006	0.10±0.006	0.10±0.006
	9	12	0.10±0.002	0.10±0.002	0.10±0.002
	12	12	0.09±0.001	0.09±0.003	0.09±0.001
	15	12	0.09±0.003	0.09±0.001	0.09±0.002

\*\* : p<0.05 from corresponding control

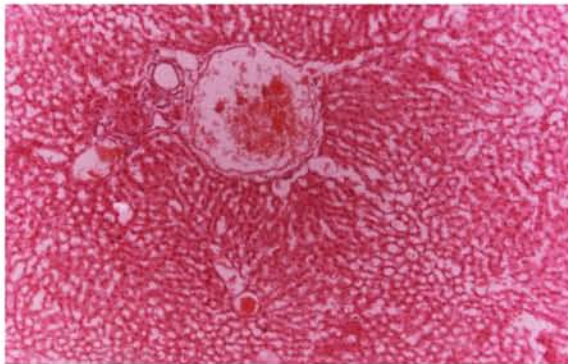


Fig. 1: Liver; Congestion and dilatation of sinusoids (H and E, 4x)

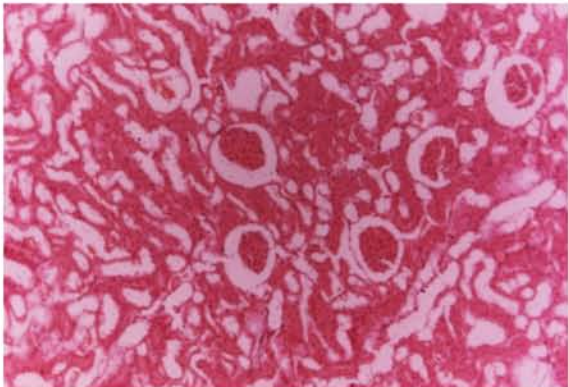


Fig. 2: Kidney; cell swelling and dilatation in renal tubules (H and E, 10x)

Creatine monohydrate is an amino acid derivative that has become a popular sports supplement used to increase muscle performance (Wyss and Kaddurah-Daouk, 2000) in humans. As creatine is taken into the muscle, it is converted to phosphocreatine, which supplies the phosphate needed for the rephosphorylation of ADP, allowing for a delay in the onset of fatigue (Persky and Brazeau, 2001).

Balsom *et al.* (1995) showed that human athletes consuming 20 g of CrM day<sup>-1</sup> for 6 day increased body mass by 1.1 kg. In the present study, feeding CrM, for 15 days had no effect on body weight gain in rats but had significant elevation in chickens (Table 1). This difference between two species is probably due to differences at the metabolism and function of creatine. It was also observed an aggressiveness in rats that is agreed with the report of Hillbrand *et al.* (1998). They found that there is a positive relationship between Creatine Kinase (CK) and aggressive behavior in 195 males.

At the present study, it was not found any significant histopathological changes in liver and kidney at creatine-supplemented groups of rats and chickens. Juhn and Tarnopolsky (1998b) reported that use of CrM at the fewer than 28 days at recommended does has not been shown to cause significant adverse effects. Poortmans and Francaux (1999) showed that neither short-term, medium-term, nor long-term oral creatine supplements induce detrimental effects on glomerular filtration rate, tubular reabsorption and glomerular membrane permeability. Mayhew *et al.* (2002) determined that oral supplementation with CrM has no long-term

detrimental effects on kidney or liver functions in highly trained college athletes. Cancela *et al.* (2007) reported that 8 week CrM supplementation does not affect on blood and urinary clinical health markers in soccer players. However these data confirm our histopathological results of liver and kidney. We also confirmed that creatine supplementation do not have side effect on muscle mass that is agreed with Bizzarini and Angelis (2004) results. They reported that no strong evidence linking creatine supplementation to deterioration of musculoskeletal functions has been found.

Taken together, the present study showed that creatine supplementation do not have any pathological effect on liver, kidney and muscle of rats and chickens and creatine can improve body weight gain in rearing chickens without any side effect.

#### ACKNOWLEDGMENTS

We are grateful to Mr. Ahmadi for preparation of pathological tissue sections. This study was supported by Payamenoor University (Esfahan branch) Research Grant.

#### REFERENCES

- Antolic, A., B.D. Roy, M.A. Tarnopolsky, R.F. Zernicke, G.R. Wohl, S.G. Shaughnessy and J.M. Bourgeois, 2007. Creatine monohydrate increases bone mineral density in young Sprague-Dawley rats. *Med. Sci. Sports Exerc.*, 39: 816-820.
- Balsom, P.D., K. Soderlund, B. Sjodin and B. Ekblom, 1995. Skeletal muscle metabolism during short duration high-intensity exercise: Influence of creatine supplementation. *Acta Physiol. Scand.*, 154: 303-310.
- Bizzarini, E. and L. De Angelis, 2004. Is the use of oral creatine supplementation safe? *J. Sports Med. Phys. Fitness*, 44: 411-416.
- Brose, A., G. Parise and M.A. Tarnopolsky, 2003. Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.*, 58: B11-B19.
- Brosnan, J.T. and M.E. Brosnan, 2007. Creatine: Endogenous metabolite, dietary and therapeutic supplement. *Annu. Rev. Nutr.*, 27: 241-261.
- Cancela, P., C. Ohanian, E. Cuitiño and A.C. Hackney, 2007. Creatine supplementation does not affect clinical health markers in soccer players. *Br. J. Sports Med.* (In Press).
- Edmunds, J.W., S. Jayapalan, N.M. DiMarco, M.H. Saboorian and H.M. Aukema, 2001. Creatine supplementation increases renal disease progression in Han: SPRD-cy rats. *Am. J. Kidney Dis.*, 37: 73-78.
- Greenhaff, P.L., 1997. The nutritional biochemistry of creatine. *J. Nutr. Biochem.*, 8: 610-618.
- Hillbrand, M., R.T. Spitz, H.G. Foster, J.H. Krystal and J.L. Young, 1998. Creatine kinase elevations and aggressive behavior in hospitalized forensic patients. *Psychiatr. Q.*, 69: 69-82.
- Ipsiroglu, O.S., C. Stromberger, J. Ilas, H. Hoger, A. Muhl and S. Stockler-Ipsiroglu, 2001. Changes of tissue creatine concentrations upon oral supplementation of creatine-monohydrate in various animal species. *Life Sci.*, 69: 1805-1815.
- Jan Groeneveld, G., J.H. Veldink, I. van der Tweel, S. Kalmijn, C. Beijer, M. de Visser, J.H. Wokke, H. Franssen and L.H. vanden Berg, 2003. A randomized sequential trial of creatine in amyotrophic lateral sclerosis. *Ann. Neurol.*, 53: 437-445.
- Juhn, M.S. and M. Tarnopolsky, 1998a. Oral creatine supplementation and athletic performance: A critical review. *Clin. J. Sport. Med.*, 8: 286-297.
- Juhn, M.S. and M. Tarnopolsky, 1998b. Potential side effects of oral creatine supplementation: A critical review. *Clin. J. Sport Med.*, 8: 298-304.
- Juhn, M.S., 1999. Oral creatine supplementation: Separating fact from hype. *Physician Sports Med.*, 27: 47-89.
- Koshy, K.M., E. Griswold and E.E. Schneeberger, 1999. Interstitial nephritis in a patient taking creatine. *N. Engl. J. Med.*, 340: 814-815.
- Kreider, R.B., C. Melton, C.J. Rasmussen, M. Greenwood, S. Lancaster, E.C. Cantler, P. Milnor and A.L. Almada, 2003. Long-term creatine supplementation does not significantly affect clinical markers of health in athletes. *Mol. Cell. Biochem.*, 244: 95-104.
- Leuzzi, V., M.C. Bianchi, M. Tosetti, C. Carducci, C.A. Cerquiglini, G. Cioni and I. Antonozzi, 2000. Brain creatine depletion: Guanidinoacetate methyltransferase deficiency (improving with creatine supplementation). *Neurology*, 55: 1407-1409.
- Mayhew, D.L., J.L. Mayhew and J.S. Ware, 2002. Effects of long-term creatine supplementation on liver and kidney functions in American college football players. *Int. J. Sport Nutr. Exerc. Metab.*, 12: 453-460.
- Mihic, S., J.R. MacDonald, S. McKenzie and M.A. Tarnopolsky, 2000. Acute creatine loading increases fat-free mass, but does not affect blood pressure, plasma creatinine, or CK activity in men and women. *Med. Sci. Sports Exerc.*, 32: 291-296.
- Parise, G., S. Mihic, D. MacLennan, K.E. Yarasheski and M.A. Tarnopolsky, 2001. Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis. *J. Applied Physiol.*, 91: 1041-1047.

- Persky, A.M. and G.A. Brazeau, 2001. Clinical pharmacology of the dietary supplement creatine monohydrate. *Pharmacol. Rev.*, 53: 161-176.
- Poortmans, J.R. and M. Francaux, 1999. Long-term oral creatine supplementation does not impair renal function in healthy athletes. *Med. Sci. Sports Exerc.*, 31: 1108-1110.
- Schilling, B.K., M.H. Stone, A. Utter, J.T. Kearney, V. Johnson, R. Coglianese, L. Smith, H.S. O'Bryant, A.C. Fry, M. Starks, R. Keith and M.E. Stone, 2001. Creatine supplementation and health variables: A retrospective study. *Med. Sci. Sports Exerc.*, 33: 183-188.
- Terjung, R.L., P. Clarkson, E.R. Eichner, P.L. Greenhaff, P.J. Hespel, R.G. Israel, W.J. Kraemer, R.A. Meyer, L.L. Spriet, M.A. Tarnopolsky, A.J. Wagenmakers and M.H. Williams, 2000. The physiological and health effects of oral creatine supplementation. *Med. Sci. Sports Exerc.*, 32: 706-717.
- Thorsteinsdottir, B., J.P. Grande and V.D. Garovic, 2006. Acute renal failure in a young weight lifter taking multiple food supplements, including creatine monohydrate. *J. Ren. Nutr.*, 16: 341-345.
- Toler, S.M., 1997. Creatine is an ergogen for anaerobic exercise. *Nutr. Rev.*, 55: 21-25.
- Walter, M.C., H. Lochmuller, P. Reilich, T. Klopstock, R. Huber, M. Hartard, M. Hennig, D. Pongratz and W. Muller-Felber, 2000. Creatine monohydrate in muscular dystrophies: A double blind, placebo-controlled clinical study. *Neurology*, 54: 1848-1850.
- Wyss, M. and R. Kaddurah-Daouk, 2000. Creatine and creatinine metabolism. *Physiol. Rev.*, 80: 1107-1213.