Effect of Guanidinoacetic acid Supplementation on Performance and Energy Utilization of Broiler Chickens (A review)

Adeniran P. O., Fagbenro R. B., Komolafe P. L., Agamugaga I. B., Bodunde T. B., Fatoki A. O., Abunsango O., Ahaiwe C., Adesanya O., Olatunji O. O., Salahudeen A. O. Usman S. O., Bamimore A. I., Babayemi O. J.
University of Ibadan, Department of Animal Science, Ibadan

Corresponding author: adeniranpaul@gmail.com

Abstract

Broiler chicken have not been able to meet their demand for energy through the de novo synthesis of creatine due to formation of creatinine resulting in creatine losses. Creatine is vital in energy metabolism by supplying phosphate groups to adenosine diphosphate and converting them to adenosine triphosphate. Creatine is synthesized through a two-step pathway in the body. Firstly, L-arginine and glycine are condensed to guanidinoacetic acid (GAA) and L-ornithine in the kidney and pancreas. A methyl group is added to GAA at the amidino group by S adenosylmethionine to form creatine through a catalyst called enzyme S-adenosyl-L methionine: N-guanidinoacetate methyltransferase. The second reaction takes place in the liver. Therefore, there is need to introduce dietary GAA which can be efficiently converted into creatine, hence increasing the creatine load in the muscle thereby leading to an improved performance and energy utilization in broiler chicken. Due to the high cost of creatine, Guanidinoacetic acid (GAA) has been considered as a cheap alternative to direct creatine supplementation. It was hypothesized that dietary guanidinoacetic acid (GAA), the precursor of creatine (Cr), would be beneficial to broilers chicken owing to improved cellular energy status and arginine sparing effects.

Effet de la Supplémentation en Acide Guanidinoacetique sur la Performance et l'Utilisation de l'Energie des Poulets de Chair (Une Revue)

Résumé

Les poulets de chair n'ont pas été en mesure de répondre à leur demande d'énergie grâce à la synthèse de novo de créatine en raison de la formation de créatinine entraînant des pertes de créatine. La créatine est vitale dans le métabolisme énergétique en fournissant des groupes phosphate à l'adénosine diphosphate et en les convertissant en adénosine triphosphate. La créatine est synthétisée par une voie en deux étapes dans le corps. Premièrement, la L-arginine et la glycine sont condensées en acide guanidinoacétique (AGA) et en L-ornithine dans les reins et le pancréas. Un groupe méthyle est ajouté à l'AGA au niveau du groupe amidino par la S adénosylméthionine pour former de la créatine grâce à un catalyseur appelé enzyme S-adénosyl-L méthionine : N-guanidinoacétate méthyltransférase. La deuxième réaction a lieu dans le foie. Par conséquent, il est nécessaire d'introduire d'AGA alimentaire qui peut être efficacement converti en créatine, augmentant ainsi la charge de créatine dans le muscle, conduisant ainsi à une amélioration des performances et de l'utilisation de l'énergie chez le poulet à griller. En raison du coût élevé de la créatine, l'acide guanidinoacétique (AGA) a été considéré comme une alternative bon marché à la *supplémentation directe en créatine. Il a été émis l'hypothèse que l'acide guanidinoacétique (AGA), le précurseur de la créatine (Cr), serait bénéfique pour les poulets de chair en raison de l'amélioration du statut énergétique cellulaire et des effets d'épargne de l'arginine.*

لم يتمكن دجاج الفروج من تلبية طلبه على الطاقة من خلال تخليق دي نوفو للكرياتين بسبب تكوين الكرياتينين مما أدى إلى خسائر الكرياتين. يعتبر الكرياتين حيويًا في استقلاب الطاقة عن طريق تزويد مجموعات الفوسفات بثنائي فوسفات الأدينوزين وتحويلها إلى ثلاثي فوسفات الأدينوزين يتم تصنيع الكرياتين من خلال مسار من خطوتين في الجسم. أولاً، يتم تكثيف ginear-L و glycine إلى حمض guanidinoacetic و ornithine-L في الكلية والبنكرياس. تتم إضافة مجموعة ميثيل إلى GAA في المجموعة الأميدية بواسطة S adenosylmethionine لتشكيل الكرياتين من خلال محفز يسمى إنزيم L -adenosyl-S guanidinoacetate methyltransferase-methionine: N. التفاعل الثاني يحدث في الكبد. لذلك، هناك حاجة لإدخال GAA الغذائي الذي يمكن تحويله بكفاءة إلى الكرياتين، وبالتالي زيادة حمولة الكرياتين في العضلات مما يؤدي إلى تحسين الأداء واستخدام الطاقة في دجاج الفروج نظرًا لارتفاع تكلفة الكرياتين، تم اعتبار حمض Guanidinoacetic (GAA) كبديل رخيص لمكملات الكرياتين المباشرة كان من المفترض أن حمض الغوانيدينوسيتيك الغذائي، سلائف الكرياتين، سيكون مفيدًا لدجاج الفروج بسبب تحسن حالة الطاقة الخلوية وتأثيرات تجنيب الأرجينين.

Introduction

In body cell processes energy in form of adenosine triphosphate (ATP) is required for maintenance and growth. Creatine phosphate is a reserve of high-energy phosphate for ATP formation (Boney et al., 2019). Creatine is a necessary component in energy metabolism, supplying phosphate groups to adenosine diphosphate (ADP) and converting them to ATP used for maintenance and growth. Creatine is not only important in energy metabolism but also plays a vital role in formation of muscles and other tissues in growing animals such as broiler chickens as observed by *Wallimann et al.* (2007). However, de novo synthesis of creatine is not able to adequately fulfil the demands of energy metabolism, especially in broiler chickens (*Delanghe et al.*, 1989; *Brosnan et al.*, 2009 in Ali, 2020) due to creatine losses in form of creatinine. Thus, *Brosnan et al.* (2009) reported that there may be a need to provide creatine to the growing tissues making the requirement for creatine proportionally greater in growing animals than in adults. *Lemme et al.* (2007a) therefore, revealed that there is a need for replacement through endogenous synthesis or intake of creatine as almost 1.7% of the creatine cycle-1 pool in an adult mammal is irreversibly converted to creatinine and excreted every day.

Due to stability issues and high cost of creatine, Guanidinoacetic acid (GAA) has been considered as an alternative to creatine supplementation (*Baker*, 2009). The GAA, the only precursor of creatine, synthesized in the kidney from glycine and arginine (Michiels et al., 2012) and the liver via the action of guanidinoacetate N-methyltransferase (Baker 2009), plays an essential role in cellular energy metabolism. The feed-grade GAA provided commercially has a better potential to be included in practical broilers' diets because it is more stable and cost-efficient than creatine and arginine (*Dilger et al.*, 2013). In a study conducted by Ale Saheb Fosoul et al. (2018), GAA inclusion in diets with lower energy content improved overall body energy retention as fat or protein, contributing to much better understanding of energy utilization in broiler chickens. Besides improving energy utilization, *Murakami* (2014) detected increase in the availability of creatine in eggs and muscle of progeny meat-type quail breeders fed dietary GAA supplementation, which results into better reproductive parameters and postnatal progeny performance. Likewise, more recent research results have shown that dietary GAA inclusion to broiler diets was reported to elevate creatine content in breast muscle (*Majdeddin et al.*, 2018; *Majdeddin et al.*, 2019), enhance muscle energy homeostasis (*DeGroot et al.,* 2019) thus, influencing energy utilization, net energy production, intestinal morphology, feed conversion ratio (FCR), and consequently growth performance of broiler chickens (*Lemme et al.* 2007b). In particular, *Arafa et al.* (2019), revealed GAA supplementation level (0.0, 0.06 and 0.12%) showing significant effect on all blood plasma parameters of broiler chickens, including levels of total protein, albumin, globulin, total lipid, triglyceride and cholesterol.

In addition, there are numerous studies conducted to investigate the effects of GAA supplementation to plant-based broiler diets with varying energy contents (*Lemme et al.,* 2007b; *Ringel et al.,* 2007; *Michiels et al.*, 2012; *Dilger et al.,* 2013; *Mousavi et al.,* 2013), whereas *Lemme et al.* (2010) concluded that creatine deficiency may be a limiting factor for performance, especially in birds-fed diets exclusively based on vegetable ingredients. *Michiels et al.,* (2012) further corroborated that this deficient may increase the requirements of arginine and glycine and amino acids necessary for creatine endogenous synthesis. However, research results on the effects of GAA on energy utilization are inconclusive necessitating the need for further study. Therefore, the objective of this study was to review the response of broiler chickens to dietary GAA supplementation on performance and energy utilization.

Broiler Chicken

Broiler is bred for meat production and could reach slaughter weight within a short period of time; between four and seven weeks (*krutchten,* 2002; *Bessei,* 2006). Barred Plymouth Rocks was one of the early pure breeds improved to produce a breeding line of all white birds; likewise, Vantress was developed from dominant white Cornish males. In 1980's leaner and higher yield broiler were produced due to continuous improvement in feed conversion and live span based on selection at a more intense level. Physiology, well-being, carcass and meat quality evaluation of broiler chickens were of current concern to breeders.

Among many other factors, feeding and housing are important factors given high consideration in the production of broiler chicken (*Odubote,* 2015). It is inferred that balanced feed and appropriate housing unit had helped broiler chicken in term of productivity, weight, and egg production. From this, it was later submitted that the Nigerian indigenous chickens could also benefit likewise if subjected to similar conditions. Broilers of both sexes are usually raised together under an intensive condition for their meat.

Apart from fast growing broilers, there are other types that get to slaughter weight at around 12 and 16 weeks of age. Those are referred to as slow growing free range and organic strain. The white feather and yellow skin colour of broiler birds were genetically analyzed and the conclusion revealed that the feather colour and the skin pigmentation were because of hybridization of domestic bird with grey jungle fowl (*Eriksson et al.,* 2008).

Broilers are known with various behaviours which make them look older than their actions. The drastic accumulation of weight made them to be slowed down in the manner they perform daily activities that involving walking or flying (locomotion), compared to other birds of lighter weight (*Week et al.,* 1994). The mystery behind the fast weight accumulation apart from the genetic factor is the high protein content of their feed. The feed conversion ratio of 1.91, 1.72 and 1.38 to a kilogram of body weight had been recorded in U.S., Canada and New Zealand commercial broiler farms (U.S. broiler performance, 2019: Ontario Canada FCR, 2015, NZ FCR, 2015).

Broiler production has made many people become economically stable while citizen of the world, collectively, had been fed with several kilogram at affordable rate and shortest possible time compare to other types of farm animals. More advantages could be derived from this poultry bird if more effort could be put into its production, especially in the developing countries of the world.

Guanidinoacetic Acid

Guanidinoacetic acid (GAA; otherwise referred to as glycocyamine or guanidinoacetate) is the natural antecedent of creatine, and it is being researched as a nutritional agent in feed. Glycocyamine (or guanidinoacetate) is a metabolite of glycine wherein the amino group has been changed over into a guanidine by guanylation (move of a guanidine bunch from arginine). In vertebrate living being, it is then converted into creatine by methylation.

Guanidinoacetic acid serves as a substrate for the biosynthesis of creatine (CREA). The phosphorylated nature of CREA helps to quickly mobilize a reserve of high energy phosphates in skeletal muscle to reuse adenosine triphosphate (ATP) and **r**efill energy levels. Studies from *Khajali et al.* (2020) showed that GAA also has numerous functions in addition to energy utilization. The feed additive has true thermal stability, which permits it to be adequately utilized in pelleted diets. GAA has high bioavailability, is cost-effective, and replaces nutritional arginine (ARG) and strength.

Digestion and absorption of GAA are comparable to other amino acids. Supplementation with 0.6–1.2 g/kg will build the muscle CREA by 14–21%, respectively. Utilization levels (retained within the body and intake) within the range of 76.2% and 100% were reported in diets deficient in arginine, while this declines with increasing arginine supplementation. (*Khajali et al.,* 2020)

Creatine is closely involved in energy metabolism through the CREA and phosphocreatine structure. It is a crucial cellular energy source for immediate regeneration of ATP to fulfill the enhanced energy demands of intense activities of tissues, especially muscle cells (*Michiels et al.*, 2012; *Zhang et al.*, 2017). It has been reported that CREA is a natural regulator of energy homeostasis, may buffer energy concentration in tissues with important and unsteady energy demands, particularly in muscles and brains (*Ostojic et al.*, 2013). Guanidinoacetic acid is the most effective immediate precursor of CREA within the body and could be naturally occurring in vertebrate animals (*Dilger et al.*, 2013).

Notably, GAA is more chemically stable and more effective than CREA at improving tissue CREA storage (*McBreairty et al.*, 2015). The GAA was reported to be more suitable than CREA as a new natural feed additive. It has also been demonstrated that diets supplemented with GAA increased the contents of CREA and phosphorus creatine in the muscle and enhanced the state of energy metabolism (*Degroot,* 2015). *Khajali et al.* (2020) proposes that a 21-day-old chicken of 985 g and average body weight with a daily weight gain of 75 g would require 169 mg of creatine.

Efficiency of Energy Utilization in Broilers

Most of the dietary energy comes from plant sources in the form of starch from cereal grains. These cereal grains provide the energy component which accounts for 60 to 70% of the nutrient requirement of poultry. In poultry research studies, Metabolizable Energy (ME) and Net Energy (NE) are usually used to measure energy availability to and utilization by the birds. Metabolizable energy can be accurately determined from the difference between the gross energy of the feed and the gross energy of excreta derived from such feeds (*NRC,* 1994). Metabolizable energy has been commonly accepted and extensively used to compare energy values of feedstuffs and diets for poultry and energy requirements are commonly expressed in this form. Net energy is a more accurate measure of energy utilization, as it measures the amount of gross energy that is used for productive purpose. It can be measured in several ways, including direct and indirect calorimetry, which are expensive. The comparative slaughter technique is cheaper and often mimics the natural rearing environment more than does calorimetry (*Sakomura et al.,* 2003)

Performance of Broiler Chicken on Different Metabolizable Energy and Balanced Protein Concentration

Amino acids and energy are requirement for skeletal muscle protein deposition, especially as glucose (*Jeyapalan,* 2007). For efficient net protein deposition there is need for balance of protein and energy at sites of protein synthesis (*Sonia et al.*, 2017). For optimum maintenance and growth of animals there is need to gratify the availability of energy in feed and feed ingredients via apparent metabolisable energy (AME) (*Sonia et al.,* 2017).

Increasing the energy and protein density of diets for broiler chickens has given inconclusive results. A pre-liminary study (*Waldroup et al.* 1990) found no effect of energy concentration of diets on growth performance or abdominal fat, although higher energy density increased dressing percentage in females, but not in males. Similarly, others found no effect of dietary energy level on carcass yield or abdominal fat (*Nunes et al.* 2012; *Duarte et al.*, 2014). In contrast, *Marcu et al.* (2012a) reported improved growth performance and carcass yield for the main cuts of broiler chickens fed diets with high energy and protein levels. *Marcu et al.* (2013) found that it reducing nutrient levels decreased protein content and elevated fat content in pectoral muscle while increasing dietary energy and protein elevated breast weight and muscle mass, and reduced fat content. In contrast, *Ferreira et al.* (1997) produced meat with less fat in broiler chickens fed low energy diets, although growth was decreased. Genotype of broiler chickens is one of the several factors that could have influenced the results of growth performance, carcass yield of cuts or meat chemical composition of broiler chickens fed diets with different nutrient density.

Maintenance Energy Requirements

The metabolizable energy maintenance (MEm) according to *National Research Council* (1994), is the needs of all body functions and moderate activity. The metabolizable energy maintenance (MEm) requirements of poultries vary widely. This variation could be attributed to genetic differences between broilers and laying hens exhibiting different growth potential and body composition. Maintenance energy requirement, as described by *Wenk et al.* (2001), is the state of the animal where its body composition remains constant and no production, work or activity done by the animal. According to *Lopez and Leeson* (2005), the maintenance energy requirement has been determined by the linear regression of energy balance measurements (that is, linear relationship between retained energy RE and ME intake). MEm represents a large portion of the metabolizable energy (ME) intake in broilers, being in the order of 42 - 44%, and is influenced by its method of expression. In growing broilers, MEm is expressed on a metabolic body weight basis, which is a function of body weight (BW) raised to 0.75 power (*Buyse et al.,* 1998). Broilers exhibit an outstanding ability to control their energy intake by adjusting their feed intake as diet energy concentration changes and the provision of dietary energy will influence the intake of all other nutrients. This difference directly influences the partitioning of energy between maintenance, fat and protein deposition and, by inference, efficiency of energy use.

As broilers grow, accretion of body fat and body protein is impacted by nutrition, bird strain, sex, environmental conditions, body weight (BW) and associated degree of maturity, and by the interaction between some or all these parameters (*Justina et al.*, 2019). Diet composition can have a major impact on carcass composition through the deposition of nutrients as fat or protein. Better knowledge of energy requirements and actual efficiencies of energy utilization for protein and fat deposition are very important to formulate diets that promote reduction in body fat deposition.

Conclusion

The current literature highlight how Guanidinoacetic acid has been considered as an alternative to creatine supplementation which can be vital in enhancing the performance and energy utilization of broiler chicken. This material is potentially safe and promising feed additives in improving the broiler performance. The role of Guanidinoacetic acid as a precursor for creatine is crucial because Guanidinoacetic acid is more chemically stable, has high bioavailability, is cost-effective, and replaces nutritional arginine. Therefore, there is need to encourage farmers to supplement GAA as feed additive in broiler diet for maximum performance and efficient utilization of energy.

References

Ale Saheb Fosoul S. S., Azarfar A., Gheisari A. and Khosravinia H. (2018). Energy utilization of broiler chickens in response to guanidinoacetic acid supplementation in diets with various energy contents. British Journal of Nutrition. 120:131–140

Ali A. C., İsmail Y., İbrahim C., Necmettin C. and Hulusi O. T. (2020). Guanidinoacetic acid supplementation is favourable to broiler diets even containing poultry by-product meal, British Poultry Science, DOI: 10.1080/00071668.2020.1720909.s

Arafa A. S. M., El-Faham A. I., Abdallah A. G., El-Sanhoury M. H. S., Ali N. G. M., Abddelaziz M. A. M., and Abdelhady A. Y. M. (2019). Effect Of Different Dietary Protein Levels with or without Guanidino Acetic Acid on Some Ileal Morphology and Blood Biochemical Parameters of Broiler Chickens. Egyptian J. Nutrition and

Feeds, 22(2) Special Issue: 235-245.

Baker D. H. (2009) Advances in protein–amino acid nutrition of poultry. Amino Acids 37, 29–41

Bessei, W. (2006). Welfare of Broilers: Science Journal. 62(3):455. Dol:10.107/s0043933960010 85.

Boney J. W., Patterson P. H. and Solis F. (2019). The effect of dietary inclusions of guanidinoacetic acid on D1-42 broiler performance and processing yields. J. Appl. Poult. Res. 29:220–228. https://doi.org/10.1016/j.japr.2019.10.008

Brosnan, J. T., Wijekoon E. P., Warford-Woolgar L., Trottier N. L., Brosnan M. E., Brunton J. A., and Bertolo R. F. P. (2009). Creatine synthesis is a major metabolic process in neonatal piglets and has important implications for amino acid metabolism and methyl balance. J. Nutr. 139:1292– 1297.

Buyse, J., Michels, H., Vloeberghs, J., Saevels, P., Aerts, J. M., Ducro, B., Berckmans, D. and Decuypere, E. (1998). Energy and protein metabolism between 3 and 6 weeks of age male broiler chicken selected for growth rate or for improved food efficiency. Br. Poult. Sci. 39: 264-272.

Degroot, A. A. (2015). *Efficacy of Dietary Guanidinoacetic Acid in Broiler Chicks. Master Degree.* Urbana IL: University of Illinois at Urbana-Champaign.

Degroot, A. A., Braun, U., and Dilger, R. N. (2019). Guanidinoacetic acid is efficacious in improving growth performance and muscle energy homeostasis in broiler chicks fed arginine-deficient or arginine-adequate diets. Poultry Science, 98:2896-2905.

Dilger, R. N., Bryant-Angeloni K., Payne R. L., Lemme A. and Parsons, C. M. (2013). Dietary guanidino acetic acid is an efficacious replacement for arginine for young chicks. Poultry Science Association Inc. 92 :171–177

Duarte KF, Junqueria OM, Borges LL, Rodrigues E, da Filardi RS, Praes MFFM, de laurentiz AC, de Domingues CHF (2014) Performance, carcass traits, and body composition of broilers fed different linseed oil levels between 21 and 56 days of age. Brazil Journal Poultry Science 16:55-60.

Eriksson, J., Larson G., Gunnarsson, U., Bed'hom, B., Tixier-Boichard, M., *et al.* (2008) Identification of the Yellow Skin Gene Reveals a Hybrid Origin of the Domestic Chicken. PLoS Genet January 23, 2008 Genetics.plosjournals.org Archived 2012-05-25 at archive.today

Farrell GD, Pinto MF, Neto MG, Ponsano EH, Goncalves CA, Bossolani IL, Pereira AG (2015) accurate adjustment of energy level in broiler chickens diet for controlling the performance and the lipid composition of meat. Cienc Rural 45:104-110

Habimana, R., Ngeno. K., Okeno, T. O., Hirwa, T. D., Tiambo, C. K. and Yao, N. K. (2021). Genome-wide Association Study of Growth Performance and Immune Response to Newcastle Disease Virus of Ingigenous Chicken in Rwanda. *Front.Genet*. 12:723980. doi: 10.3389/fgene. 2021.723980

Jorgensen, H., P. Sorensen, and B. O Eggum. (1990). Protein and energy metabolism in broiler chickens selected for either body weight gain or feed effificiency. Br. Poult. Sci. 31:517–524.

Justina V. C., Nirun Boonsinchai, J. W., Judith A. E., Craig N. C. (2019). The dynamics of body composition and body energy content in broilers, Poultry Science, Vol 98:2. 866-877

Khajali F., Lemme A. & Rademacher-

Heilshorn M. (2020) Guanidinoacetic acid as a feed supplement for poultry, World's Poultry Science Journal, 76:2, 270-291.

Kruchten, T. (2002). U.S Broiler Industry Structure. National Agricultural Statistics Services (NASS), Agricutural Statistics Board, U.S. Department of Agriculture, Archived from the original. Retrived June 23, 2012.

Lemme, A., Gobbi R., Helmbrecht A., Van Der Klis J. D., Firman, J., Jankowski J. and Kozlowsk K. (2010). Use of guanidino acetic acid in all-vegetable diets for turkeys. Proc. 4th Turkey Sci. Prod. Conf., Macclesfield, UK. Turkeytimes, Tarpor. Pages 57–61

Lemme, A., J. Ringel, A. Sterk, and J. F. Young. (2007). Supplemental guanidino acetic acid affects energy metabolism of broilers. Pages 339–342 in Proc. 16th Eur. Symp. Poult. Nutr., Strasbourg, France. World's Poult. Sci. Assoc., Beekbergen, the Netherlands.

Lemme, A., J. Ringel, H. S. Rostagno, and M. S. Redshaw. (2007). Supplemental guanidino acetic acid improved feed conversion, weight gain, and breast meat yield in male and female broilers. Pages 335–338 in Proc. 16th Eur. Symp. Poult. Nutr., Strasbourg, France. World's Poult. Sci. Assoc., Beekbergen, the Netherlands.

Lopez, G. and Leeson, S. (2005). Utilization of metabolizable energy by young broilers and birds of intermediate growth rate. Poult. Sci. 84: 1069-1076.

Majdeddin, M., Golian, A., Kermanshahi, H., De Smet, S., and Michiels, J. (2018). Guanidinoacetic acid supplementation in broiler chickens fed on cornsoybean diets affects performance in the finisher period and energy metabolites in breast muscle independent of diet nutrient density. British Poultry Science, 59: 443-451.

Majdeddin, M., Golian, A., Kermanshahi, H., Michiels, J., and De Smet, S. (2019). Effects of methionine and guanidinoacetic acid supplementation on performance and energy metabolites in breast muscle of male broiler chickens fed corn-soybean diets. British Poultry Science, 60, 554-563

Marcu A, Vacaru-Opris I, Marcu A, Nicula M, Dronca D, Kelciov B (2012a) Effect of different levels of dietary protein and energy on the growth and slaughter performance at "Hybro PN+" broiler chickens. Paper Animal Science Biotechnology 46:333-340

Marcu A, Vacaru-Opris I, Marcu A, Nicula M, Dronca D, Kelciov B (2013) Effect of diets with different energy and protein levels on breast muscle characteristics of broiler chickens. Paper Animal Science Biotechnology 46:333-340

McBreairty, L. E., Robinson, J. L., Furlong, K. R., Brunton, J. A., and Bertolo, R. F. (2015). Guanidinoacetate is more effective than creatine at enhancing tissue creatine stores while consequently limiting methionine availability in Yucatan miniature pigs. *PLoS One* 10:e0131563. doi: 10.1371/journal.pone.0131563

Michiels, J., L. Maertens, J. Buyse, A. Lemme, M. Rademacher, N. A. Dierick, and S. De Smet. (2012). Supplementation of guanidinoacetic acid to broiler diets: Effects on performance, carcass characteristics, meat quality, and energy metabolism. Poult. Sci. 91:402–412.

Mousavi, S. N., Afsar A., and Lotfollahian H. (2013). Effects of guanidinoacetic acid supplementation to broiler diets with varying energy contents. J. Appl. Poult. Res. 22:47–54.

Murakami A. E., Rodrigueiro R. J. B.,

Santos T. C., Ospina-Rojas I. C. and M. Rademacher. (2014). Effects of dietary supplementation of meat-type quail breeders with guanidinoacetic acid on their reproductive parameters and progeny performance. Poultry Science Association Inc. 93 :2237–2244.

National Research Council (NR. Effect of environment on nutrient requeriments of domestic animals. National Academy of Sciences, Washington, DC; 1981.

NRC., (1994). Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC., USA

Nunes J. O, Bertechni AG, de Brito JAG, Makiyama L, Mesquita FR, Nishio CM (2012) Evaluation of cysteamine associated with different energy patterns in diets for broilers chickens. Rev Bras Zootec 41:1965-1960

Odubote, K. (2015). The Local Chicken of Nigeria. www.researchgate.net/publication/276058544. doi:10.13140/RG2.1.2193.5520

Ostojic, S. M., Niess, B., Stojanovic, M., and Obrenovic, M. (2013). Creatine metabolism and safety profiles after six-week oral guanidinoacetic acid administration in healthy humans. *Int. J. Med. Sci.* 10, 141–147.

Sakomura, N. K., R. Silva, H. P. Couto, C. Coon and C. R. Pacheco, (2003). Modeling metabolizable energy utilization in broiler breeder pullets. Poult. Sci., 82: 419-427.

Waldroup PW, Tidwell NW, Izat AL (1990) The effects of energy and amino-acid levels on performance and carcass quality of male and female broilers grown seperately. Poultry Science 69:1513-1521

Wallimann, T., M. Tokarska-Schlattner, D. Neumann, R. M. Epand, R. F. Epand, R. H. Andres, H. R. Widmer, T. Hornemann, V. A. Saks, I. Agarkova, and U. Schlattner. (2007). The phosphocreatine circuit: Molecular and cellular physiology of creatine kinases, sensitivity to free radicals, and enhancement by creatine supplementation. Pages 195– 264 in Molecular System Bioenergetics: Energy for Life. V. Saks, ed. Wiley-VCH, Weinheim, Germany.

Weeks, C. A.; Nicol, C. J.; Sherwin, C. M.; Kestin, S.C. (1994). "Comparison of the behaviour of broiler chickens in indoor and free-range environments". Animal Welfare. 3: 179 192.

Wenk C. Summary of the discussion. Energy Metabolism of Farm Animals. In: McCracken KJ, Unsworth EF, Wylie ARG, Eds. Proceedings of the 14th Symposium on Energy Metabolism, Newcastle, Co. Down, Northern Ireland,1997. University Press, Cambridge, p. 265-267.

Zhang, L., Wang, X., Li, J., Zhu, X., Gao, F., and Zhou, G. (2017). Creatine monohydrate enhances energy status and reduces glycolysis via inhibition of AMPK pathway in pectoralis major muscle of transport-stressed broilers. *J. Agric. Food Chem.* 65, 6991–6999.