



## Preclinical Interpretation of Oral Urolithin-A Against Respiratory Disease Among Calves: Unlocking The "Gut-Lung Axis"

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### ABSTRACT

Pomegranate, as a plant, has long been known for its rich content of ellagitannins. Ellagitannins are completely hydrolyzed to ellagic acid in the intestine, which then gives rise to urolithins. This natural compound may support pulmonary system health as a feed additive, not a pharmacological agent. The aim of the present study was to describe the efficacy of urolithin-a against 17 Holstein calves with respiratory disease. Written owner consent and ethical guidelines report were considered available. No etiological investigation was conducted (outside the purpose of this study, as it was a preclinical, not clinical study). With the support of 3 veterinary experts, respiratory disease among calves was diagnosed based on the University of Wisconsin Calf Health Scoring Schedule (WIN score). Urolithin-a compound was administered perorally to diseased calves at 3 mg/kg for 10 consecutive days. No adverse effects were observed during feed additive administration. WIN scores were investigated before (day 0) and after treatment (day 10). Mean (8.24 vs. 1.82) and median (8.00 vs. 2.00) WIN scores were decreased in association with urolithin-a treatment before and after treatment, respectively ( $p < 0.0001$ ). In this preclinical setting, it would not be unfair to suggest that urolithin-a should help in the regression of respiratory disease among the enrolled calves. This nutraceutical administered via the gastrointestinal route was able to reverse respiratory signs indicating the secrets of the 'gut-lung axis' in this field study. The results obtained should be able to change treatment protocols.

### INTRODUCTION

Plants such as pomegranate (Cerde et al., 2004) and other relevant ones have high ellagitannin content. Hydrolyzation of ellagitannins into ellagic acid exists within the intestinal tract in which then urolithins are converted by metabolization. Productive concentration depends on the phenotypes of metabolism and diminishes with aging (Cortés-Martín et al., 2020). Moreover furthermore, ellagitannins/ellagic acid metabolic activity have been modified through gut microbiota composition. As an example, *Bifidobacterium pseudocatenulatum* INIA P815 (Gaya et al., 2018) and *Enterococcus faecium* FUA027 (Zhang et al., 2022) exhibited urolithin A, while

*Ellagibacter isourolithifaciens* DSM104140T (Selma et al., 2017; Beltrán et al., 2018) and *Gordonibacter urolithinifaciens* DSM 27213 T presented isourolithin A/urolithin C forms of ellagic acid (Watanabe et al., 2020). Urolithin production through ellagitannins has been declared among several different animals. In cattle and sheep, the foremost detectable urolithin compounds were isourolithin A, and urolithin B, whereas urolithin A was generally exhibited thorough intestinal route (Espin et al., 2013). Given some suggested data (González-Barrio et al., 2012, Alic Ural et al., 2025) is available for urolithin a among cattle, its efficacy has not been validated through gut-lung axis among calves. In the present study the hypothesis was, to determine the efficacy of urolithin A

given in oral route interacts with respiratory disease symptoms. Therefore, in the present study the purpose was to elucidate the efficacy of urolithin-against respiratory disease among calves, further evidence of proof for gut-lung axis.

## MATERIALS AND METHODS

### Animals and Procedures

Holstein calves (n=17) at the age of 21 to 58 days, of both sexes, were clinically evaluated for respiratory disease (relevant clinical signs) by 3 veterinary surgeons at the same time, individually. In the present study no healthy control group was enrolled due to limited access and

permission by the owners. In an attempt to diagnose respiratory disease among calves previously determined, relatively summarized and novel, WIN score (Jaureguiberry et al., 2023) (Table 1) was based on 4-level scoring scales involving nasal (1)/ocular (2) discharge, coughing (3), ear position (4) and rectal temperature (5) (Table 1). Involved calves were classified positive for respiratory disease even if the aggregate score was  $\geq 5$ . Exclusion criteria were enrolled as: 1) evidence of comorbidity diseases, 2) recent vaccination or drug prescription, 3) and if there exists no evidence of respiratory signs.

**Table 1.** Even if a calf with respiratory disease exhibited the aggregate score was  $\geq 5$ , diagnosis was fully supported, based on WIN score (Jaureguiberry et al., 2023).

WIN score for diagnosing respiratory disease (9)	0	1	2	3
nasal discharge (n=)	normal serous discharge	Few amount of unilateral cloudy discharge	bilateral overload discharge	cloudy/ mucus mucopurulent discharge
ocular discharge	0 = normal	1 = few ocular discharge	2 = moderate bilateral ocular discharge	3 = heavy ocular discharge
coughing	0 = none	1 = induced single cough	2 = induced repeated coughs/ or occasional spontaneous coughs	3= repeated spontaneous coughs
ear position	0 = normal	1 = ear flick or head shake	2 = slight unilateral droop	3= head tilt or bilateral droop
rectal temperature	0 = 37.8 to 38.2°C	1 = 38.3 to 38.8°C	2 = 38.9 to 39.4°C	3 = > 39.4°C)

<sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.0001$ .

This study was performed with written owner consent and confirmed by Aydın Adnan Menderes University, Local Ethics Committee for Animal Researches (HADYEK) with report no: 64583101/2024/119.

### Urolithin-a Preferred at This Study

DQOI Urolithin A Liquid Drops 3000 mg was used at a dosage of 3 mg/kg p.o. Each calf was well accepted and there were no side effects. The drop was safely used perorally with the -help of veterinary surgeons with HADYEK Certificates. During trial not other nutraceutical or any drug were used. There were no side effects noticed through usage of oral route. All calves accepted well.

### Statistical Analyses

Statistical analyses were conducted using SPSS 29.0 (IBM, USA). Descriptive statistics, including means, medians, and standard errors, were calculated for pre-treatment and post-treatment groups. The Wilcoxon test was used to evaluate differences between pre and post treatment groups. The p value less than 0,05 was considered significant in all analyses.

## RESULTS

As was also declared at materials and methods section, whether a calf with respiratory disease presented an aggregate score was  $\geq 5$ , diagnosis was made through WIN score (Jaureguiberry et al., 2023). For this sense; in a total of 17 calves WIN Scores were ranged between 5 to 12

prior to treatment, whereas following urolithin-a intervention given by oral route, scores altered between 0 to 4 (at most). Interestingly 4 calves exhibited scores 0, and other relevant 4 ones as 1 after urolithin-a prescription. Regarding WIN Scores mean values were 8.24 vs. 1.82, following urolithin-a treatment, respectively (Table 2).

## DISCUSSION AND CONCLUSION

Urolithin-A was able to suppress lung inflammation, oxidative stress, and apoptosis in acute lung injury in mice (Jiao et al., 2024). Moreover in a prior study has already been elucidated as a treatment target against acute lung injury in mice (Lou et al., 2023). In a prior and interesting research urolithin-A was searched for its efficacy against pulmonary hypertension to those of mice were exposed to hypoxia. In that study urolithin-A alleviated progression of pulmonary hypertension through inhibition of PASM pyroptosis, indicating the latter natural compound's treatment value (He et al., 2024). Furthermore urolithin-A was able to induce preventive autophagy for suppressing inflammatory respond, oxidation and relevant endoplasmic reticulum stress to those of 1-week-old C57BL/6 mice with pediatric pneumonia (Cao et al., 2022). In the present study urolithin-a significantly decreased ( $p < 0.0001$ ) WIN Scores (Table 2).

In general urolithin-a is the vast majority biologically active and investigated one in contrast to other relevant urolithin species, prominent with several efficacy [i.e. anti-

oxidant, anti-inflammatory, anti-cancer and anti-aging (Lee et al., 2021; Luan et al., 2021; Vini et al., 2023; Huang et al., 2023). Prior investigations denoted that urolithin-a was capable of suppressing NLRP3 inflammasome activation (Tao et al., 2021; Zhang et al., 2021), which potentially alters inflammation. Nevertheless, the therapeutic potential of urolithin a in respiratory disease among calves has not been established. This study reported herein, could thus be the first one investigating the efficacy of urolithin-a as a feed additive prevention and probably curing respiratory disease in a preclinical setting.

Oak leaves involve certain levels of hydrolyzable tannins [composed of gallotannins and ellagitannins] (Salminen et al., 2004). It has been suggested that elevated levels of tannins exhibited in forage inhibited bacteria among intestinal location and diminished ruminant performance, throughly via deducing intake and nutrient digestibility (Smith et al., 2005). It has been postulated that, tannins are capable of exhibiting both adverse and beneficial efficacy among ruminants, which is linked to type/volume nourished (Makkar et al., 1988; Shabtay et al., 2008). Both ellagitannins and ellagic acid paid great attention lately, as because of their antioxidant efficacy (Fukuda et al., 2003; Gil et al., 2000) Elagitannin-rich diets improved plasma lipids, diminished oxidative stress and induced apoptosis all were linked to declined risk for several chronic diseases (Aviram et al., 2000; Larossa et al., 2007; Heber, 2008). All aforementioned efficacy have throughly been dedicated to urolithins, well metabolites of ellagitannins exhibited via microbiota (Cerdeja et al., 2004, 2005; Gonzalez-Sarrias et al., 2010).

In a prior study it was nominated that ellagitannins were subjected to metabolization in rumen to urolithins. Several urolithins were investigated among ruminal fluid, plasma, urine and feces. Oak leaf ellagitannins declined as they were entirely transformed into urolithins [the vast majority isourolithin A/urolithin B], through rumen/gut microbiota. Finally foremost urolithins exhibited among cattle were detected as isourolithin A and urolithin B (González-Barrio et al., 2012). In the present study we did not have the possibility of detecting metabolites and gut microbiota alterations among calves enrolled. Apart from this disadvantage we had some limitations. Detailed ultrasonographic examinations (Aliç Ural and Ural, 2023), serum zonulin levels (Aliç Ural and Ural, 2023), fractional exhaled nitric oxide tests were lacking, which would have helped both supporting diagnostic and prognostic portions, even if performed.

In the present study commercially available urolithin-a (DQOI Urolithin A Liquid Drops 3000 mg) was given p.o. to calves with respiratory disease without any side effects. Feed additive was well accepted. Mean (8.24 vs. 1.82) and median (8.00 vs. 2.00) WIN Scores were declined ( $p < 0.0001$ ) in respond to urolithin-a treatment prior to and thereafter, respectively. This novel and interested nutraceutical as given perorally (through gastrointestinal tract) was able to withdraw respiratory signs among calves, indicating proof of 'gut-lung axis' in this preclinical setting, field study. Available data herein, might have helped supportive treatment-regimes.

#### Acknowledgement

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#### Ethical Declaration

This study was performed with written owner consent and confirmed by Aydın Adnan Menderes University, Local Ethics Committee for Animal Researches (HADYEK) with report no: 64583101/2024/119.

#### Conflict of Interest

The authors declare that they have no competing interests.

#### Authorship contributions

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#### REFERENCES

- Aliç Ural D, Ural K. 2023. Zonulin as a Preliminary Biomarker of Lung Permeability Among Diseased Calves: Cohort Study. *Egyptian Journal of Veterinary Sciences*, 54(4), 601-607.
- Aliç Ural D, Ural K, Paşa S, Gültekin M, Erdoğan H, Erdoğan S, Balıkcı C, Kızılkant İ. 2025. Investigation Of The Effectiveness Of Urolithin-A On Fecal Scores In Calves With Non-Infectious Diarrhea. *Current Perspectives on Health Sciences*, 6(1), 39-46
- Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, Hayek T, Presser D, Fuhrman B. 2000. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *The American Journal of Clinical Nutrition*, 71(5), 1062-1076.
- Beltrán D, Romo-Vaquero M, Espín JC, Tomás-Barberán FA, Selma MV. 2018. *Ellagibacter isourolithinifaciens* gen. nov., sp. nov., a new member of the family Eggerthellaceae, isolated from human gut. *International Journal of Systematic and Evolutionary Microbiology*, 68(5), 1707-1712.
- Cao X, Wan H, Wan H. 2022. Urolithin A induces protective autophagy to alleviate inflammation, oxidative stress, and endoplasmic reticulum stress in pediatric pneumonia. *Allergologia et Immunopathologia*, 50(6), 147-153.
- Cerdá B, Espín JC, Parra S, Martínez P, Tomás-Barberán FA. 2004. The potent in vitro antioxidant ellagitannins from pomegranate juice are metabolised into bioavailable but poor antioxidant hydroxy-6H-dibenzopyran-6-one derivatives by the colonic microflora of healthy humans. *European Journal Of Nutrition*, 43, 205-220.
- Cerdá B, Tomás-Barberán FA, Espín JC. 2005. Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability. *Journal of Agricultural and Food Chemistry*, 53(2), 227-235.
- Cortés-Martín A, Selma MV, Tomás-Barberán FA, González-Sarrias A, Espín JC. 2020. Where to look into the puzzle of polyphenols and health? The postbiotics and gut microbiota associated with human metabolotypes. *Molecular Nutrition & Food Research*, 64(9), 1900952.
- Espín JC, Larrosa M, García-Conesa MT, Tomás-Barberán F. 2013. Biological significance of urolithins, the gut microbial ellagic acid-derived metabolites: the

evidence so far. *Evidence-Based Complementary and Alternative Medicine*, 2013(1), 270418.

Fukuda T, Ito H, Yoshida T. 2003. Antioxidative polyphenols from walnuts (*Juglans regia* L.). *Phytochemistry*, 63(7), 795-801.

Gaya P, Peirotén Á, Medina M, Álvarez I, Landete JM. 2018. *Bifidobacterium pseudocatenulatum* INIA P815: The first bacterium able to produce urolithins A and B from ellagic acid. *Journal of Functional Foods*, 45, 95-99.

Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, 48(10), 4581-4589.

González-Barrio R, Truchado P, García-Villalba R, Hervás G, Frutos P, Espín JC, Tomás-Barberán FA. 2012. Metabolism of oak leaf ellagitannins and urolithin production in beef cattle. *Journal of Agricultural and Food Chemistry*, 60(12), 3068-3077.

González-Sarrías A, Giménez-Bastida JA, García-Conesa MT, Gómez-Sánchez MB, García-Talavera NV, Gil-Izquierdo A, Sánchez-Alvarez C, Fontana-Compiano LO, Morga-Egea JP, Pastor-Quirante FA, Martínez-Díaz F, Tomás-Barberán FA, Espín JC. 2010. Occurrence of urolithins, gut microbiota ellagic acid metabolites and proliferation markers expression response in the human prostate gland upon consumption of walnuts and pomegranate juice. *Molecular Nutrition & Food Research*, 54(3), 311-322.

He X, Wu Z, Jiang J, Xu W, Yuan A, Liao F, Ding S, Pu J. 2024. Urolithin A protects against hypoxia-induced pulmonary hypertension by inhibiting pulmonary arterial smooth muscle cell pyroptosis via AMPK/NF- $\kappa$ B/NLRP3 signaling. *International Journal of Molecular Sciences*, 25(15), 8246.

Heber D. 2008. Multitargeted therapy of cancer by ellagitannins. *Cancer Letters*, 269(2), 262-268.

Huang JR, Zhang MH, Chen YJ, Sun YL, Gao ZM, Li ZJ, Zhang GP, Qin XY, Dai XY, Yu XY, Wu XQ. 2023. Urolithin A ameliorates obesity-induced metabolic cardiomyopathy in mice via mitophagy activation. *Acta Pharmacologica Sinica*, 44(2), 321-331.

Jaureguiberry M, Rearte R, Marconi MJ, Giuliodori MJ, Madoz LV, Pinedo FA, de la Sota RL. 2023. A simplified scoring system for the diagnosis of diarrhea and respiratory diseases in dairy calves. *The Canadian Veterinary Journal*, 64(6), 553-557.

Jiao P, Wang Y, Ren G, Chu D, Li Y, Yang Y, Sang T. 2024. Urolithin A exerts a protective effect on lipopolysaccharide-induced acute lung injury by regulating HMGB1-mediated MAPK and NF- $\kappa$ B signaling pathways. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 397(8), 5765-5777.

Larrosa M, Tomás-Barberán FA, Espín JC. 2006. The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. *The Journal of Nutritional Biochemistry*, 17(9), 611-625.

Lee HJ, Jung YH, Choi GE, Kim JS, Chae CW, Lim JR, Kim SY, Yoon JH, Cho JH, Lee SJ, Han HJ. 2021. Urolithin A suppresses high glucose-induced neuronal amyloidogenesis by modulating TGM2-dependent ER-

mitochondria contacts and calcium homeostasis. *Cell Death & Differentiation*, 28(1), 184-202.

Lou L, Wang M, He J, Yang S, Meng F, Wang S, Jin X, Cai C. 2023. Urolithin A (UA) attenuates ferroptosis in LPS-induced acute lung injury in mice by upregulating Keap1-Nrf2/HO-1 signaling pathway. *Frontiers in Pharmacology*, 14, 1067402.

Luan P, D'Amico D, Andreux PA, Laurila PP, Wohlwend M, Li H, de Lima TI, Place N, Rinsch C, Zanou N, Auwerx J. 2021. Urolithin A improves muscle function by inducing mitophagy in muscular dystrophy. *Science Translational Medicine*, 13(588), eabb0319.

Makkar HP, Singh B, Dawra RK. 1988. Effect of tannin-rich leaves of oak (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. *British Journal of Nutrition*, 60(2), 287-296.

Salminen JP, Roslin T, Karonen M, Sinkkonen J, Pihlaja K, Pulkkinen P. 2004. Seasonal variation in the content of hydrolyzable tannins, flavonoid glycosides, and proanthocyanidins in oak leaves. *Journal of Chemical Ecology*, 30, 1693-1711.

Selma MV, Beltrán D, Luna MC, Romo-Vaquero M, García-Villalba R, Mira A, Espín JC, Tomás-Barberán FA. 2017. Isolation of human intestinal bacteria capable of producing the bioactive metabolite isourolithin A from ellagic acid. *Frontiers in Microbiology*, 8, 1521.

Shabtay A, Eitam H, Tadmor Y, Orlov A, Meir A, Weinberg P, Chen Y, Brosh A, Izhaki I, Kerem Z. 2008. Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as a novel beef cattle feed. *Journal of Agricultural and Food Chemistry*, 56(21), 10063-10070.

Smith AH, Zoetendal E, Mackie RI. 2005. Bacterial mechanisms to overcome inhibitory effects of dietary tannins. *Microbial Ecology*, 50, 197-205.

Tao H, Li W, Zhang W, Yang C, Zhang C, Liang X, Yin J, Bai J, Ge G, Zhang H, Yang X, Li H, Xu Y, Hao Y, Liu Y, Geng D. 2021. Urolithin A suppresses RANKL-induced osteoclastogenesis and postmenopausal osteoporosis by, suppresses inflammation and downstream NF- $\kappa$ B activated pyroptosis pathways. *Pharmacological Research*, 174, 105967.

Vini R, Jaikumar VS, Remadevi V, Ravindran S, Azeez JM, Sasikumar A, Sundaram S, Sreeja S. 2023. Urolithin A: A promising selective estrogen receptor modulator and 27-hydroxycholesterol attenuator in breast cancer. *Phytotherapy Research*, 37(10), 4504-4521.

Watanabe H, Kishino S, Kudoh M, Yamamoto H, Ogawa J. 2020. Evaluation of electron-transferring cofactor mediating enzyme systems involved in urolithin dehydroxylation in *Gordonibacter urolithinifaciens* DSM 27213. *Journal of Bioscience and Bioengineering*, 129(5), 552-557.

Zhang X, Fang Y, Yang G, Hou X, Hai Y, Xia M, He F, Zhao Y, Liu S. 2022. Isolation and characterization of a novel human intestinal *Enterococcus faecium* FUA027 capable of producing urolithin A from ellagic acid. *Frontiers in Nutrition*, 9, 1039697.

Zhang Y, Aisker G, Dong H, Halemahebai G, Zhang Y, Tian L. 2021. Urolithin A suppresses glucolipotoxicity-induced ER stress and TXNIP/NLRP3/IL-1 $\beta$  inflammation signal in pancreatic  $\beta$  cells by regulating AMPK and autophagy. *Phytomedicine*, 93, 153741.