

Antioxidative effect of mistletoe leaf meal supplemented diets in laying pullets

Jimoh, O.A.^{1,2@}; Ihejirika, U.G.²; Balogun, A.S.³ and Uwaeziozi U.C.²

¹Agricultural Technology Department, Federal Polytechnic Ado Ekiti, Ekiti State, Nigeria.

²Animal Physiology unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

³Department of Animal Health and Production Technology, Oyo State College of Agriculture, Igboora, Oyo State, Nigeria.

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Correspondencia a los autores/Contact e-mail:
abubakarjimoh2011@gmail.com

SUMMARY

The present study was conducted to examine the antioxidant potential of African mistletoe (*Viscum album* L.) as alternative feed additive for Laying Pullets. Fresh African mistletoe leaves were harvested from cocoa trees and shade dried to constant weight. The leaves were grounded and were designated as mistletoe leaf meal (AMLM). Total of sixty ISA Brown pullets at 18 weeks old were randomly allocated to four dietary treatment consisting of 5 replicates with 3 birds per replicate, when egg production was 4% in a completely randomized design. The birds were fed layer ration and dietary mistletoe supplement as treatments T1 (basal diet + 0% AMLM), T2 (basal diet + 2% AMLM), T3 (basal diet + 4% AMLM) and T4 (basal diet + 6% AMLM) during 16 week feeding trial. At 8th week and end of the feeding trial, blood samples were collected through the Jugular vein into a sample bottle from all birds for serum oxidative indicator assay; malondialdehyde (mMDA/mgprotein), total antioxidant activity (mmol/litre), glutathione peroxidase (GPx, $\mu\text{gGSH}/\text{min}/\text{mgprotein}$), superoxide dismutase (SOD, U/min/mg protein) and catalase ($\text{nmH}_2\text{O}_2/\text{min}/\text{mg protein}$) using standard procedures. The result obtained revealed that at 8 weeks of lay, lipid peroxidation in laying pullets were significantly ($P<0.05$) lowered by mistletoe supplementation while total antioxidant activity of significantly ($P<0.05$) increased with mistletoe inclusion. Pullets fed 6% mistletoe supplemented diets significantly ($P<0.05$) enhanced catalase and glutathione peroxidase activity. Total antioxidant activity and all antioxidant enzymes of laying pullets increased, as laying cycle progresses, while lipid peroxidation reduced. This could signify that laying pullets have better antioxidative stability at 16 weeks of lay compared than pullets at 8 weeks of lay. It can be concluded that African mistletoe leaf meal inclusion in laying pullets diet enhance antioxidant profile in pullets.

Efecto antioxidativo de las dietas suplementadas de las comidas de hoja de mantequilla en las pulletas de colocación

RESUMEN

El presente estudio se llevó a cabo para examinar el potencial antioxidante del muérdago africano (*Viscum album* L.) como aditivo alimenticio alternativo para las gallinas ponedoras. Las hojas frescas del muérdago africano fueron cosechadas de los árboles del cacao y la cortina secó al peso constante. Las hojas se pusieron a tierra y se designaron como harina de muérdago (AMLM). Se asignaron aleatoriamente a un total de sesenta pollos marrones ISA a las 18 semanas de edad a cuatro tratamientos dietéticos consistentes en 5 repeticiones con 3 aves por repetición, cuando la producción de huevos fue del 4% en un diseño completamente al azar. Las aves fueron alimentadas con raciones de raciones y suplementos de muérdago dietético como tratamientos T1 (dieta basal + 0% AMLM), T2 (dieta basal + 2% AMLM), T3 (dieta basal + 4% AMLM) y T4 (dieta basal + 6% AMLM) durante el ensayo de alimentación de 16 semanas. A la octava semana y al final del ensayo de alimentación, se recogieron muestras de sangre a través de la vena yugular en una botella de muestras de todas las aves para el ensayo de indicador oxidativo sérico; malondialdehído (mMDA / mgprotein), actividad antioxidante total (mmol / litro), glutatión peroxidasa (GPx, $\mu\text{gGSH} / \text{min} / \text{mgprotein}$), superóxido dismutasa (SOD, U / min / mg de proteína) y catalasa ($\text{nmH}_2\text{O}_2 / \text{min} / \text{mg de proteína}$) utilizando procedimientos estándar. El resultado obtenido reveló que a las 8 semanas de puesta, la peroxidación lipídica en las gallinas ponedoras se redujo significativamente ($P<0,05$) por la suplementación de muérdago, mientras que la actividad antioxidante total de significativamente ($P<0,05$) aumentó con la inclusión de muérdago. Las pollas alimentadas con 6% de dietas suplementadas con muérdago aumentaron significativamente ($P<0,05$) la actividad de la catalasa y glutatión peroxidasa. La actividad antioxidante total y todas las enzimas antioxidantes de las gallinas ponedoras aumentaron a medida que el ciclo de postura progresó, mientras que la peroxidación lipídica disminuyó. Esto podría significar que las pollitas ponedoras tienen una mejor estabilidad antioxidante a las 16 semanas de puesta en comparación con las pollas a las 8 semanas de puesta. Se puede concluir que la inclusión de las hojas de muérdago africanas en la dieta de pollos ponedoras mejora el perfil antioxidante en las pollas.

INTRODUCTION

Medicinal plants have been a readily available source of drugs since ancient times and even today almost 50% of the new drugs have been patterned after phytochemicals (Papuc et al., 2010). Recognizing the medicinal significance of indigenous plants, World Health Organization (WHO), in its 1997 guideline, states that "effective locally available plants can be used as substitutes for drugs" (Papuc et al., 2010). Mistletoe (*Viscum album* L.) is abundant in forest regions and orchards as a hemiparasite and has long been used in animal diets especially during droughts and winters (Hossain et al., 2012). In comparison to commonly used conventional forages, mistletoe contains low protein, moderate fiber, and is high in minerals; therefore it can provide alternative mineral and forage sources for ruminant feeding (Hossain et al., 2012; Madibela et al., 2000; Alemede et al., 2014). The main constituents of mistletoe are lectins (mistletoe Lectins I, II, III), viscotoxins, polysaccharides, cyclitols, flavonoids, phenyl propane derivatives, triterpenoids like amyryl, betulonic acid, oleanolic acid, phytosterols, amino acids, alkaloids, cyclic peptides, histamine, acetylcholine, and 9.3% protein (EMEA 2000). Previous studies have demonstrated that extracts from this plant possess pharmacological properties having immunomodulatory, anti-inflammatory, cardiovascular, and antimicrobial effects (Hossain et al., 2012; Kienle and Kiene 2003; Alison et al., 2000). Nwaegerue et al. (2007) observed a glucose lowering effect in normal and diabetic rats using leaf extracts of *V. album* (Hossain et al., 2012). Shi et al. (2006) found that some compositions of Mistletoe (Mistletoe alkali) can act effectively *in vitro* as antioxidants and peroxy radical scavengers.

Oxidative stress, defined as an imbalance between oxidants and antioxidants in favor of oxidants, leads to multiple biochemical changes in animal and human organism, that are causative factor of several chronic diseases, such as cardiovascular diseases, mutagenesis and cancer, several neurodegenerative disorders and aging process (Papuc et al. 2010). Endogenous antioxidants in medicinal herbs may play an important role in antioxidative defense against oxidative damage, possibly protecting the biological functions of cells (Shi et al., 2006). There is increasing interest in the protective and biological function of natural antioxidants contained in herbs, which are candidates for the prevention of oxidative damage. There is dearth of information on effects of this plant on oxidative status of laying pullets. Therefore, this experiment was designed to evaluate the effect of African mistletoe on serum oxidative status of pullets.

MATERIALS AND METHODS

The study was carried out at the poultry unit, Department of Agricultural Technology of the Federal polytechnic Ado Ekiti, Ekiti State, Nigeria. The study was approved by our institutional committee on the care and use of animals for research.

Fresh African mistletoe leaves were harvested from cocoa trees and shade dried to constant weight. The

leaves were grounded and were designated as African mistletoe leaf meal (AMLM). Sixty ISA Brown pullets of 18 weeks old were used in the study. The pullets were housed in battery cage, each treatment consist of five replicates, each replicate consist of three birds and were randomly allocated to four dietary treatment when egg production was 4% in a completely randomized design. The birds were fed layer ration and dietary mistletoe supplement as treatments T1 (basal diet + 0% AMLM), T2 (basal diet + 2% AMLM), T3 (basal diet + 4% AMLM) and T4 (basal diet + 6% AMLM), respectively during 16 week feeding trial. All diets were formulated to meet or exceed the nutrient requirements of laying pullets (NRC 1994).

At 8 and 16 weeks of the feeding trial, blood was collected through the Jugular vein into sample bottle from all birds, serum was separated by centrifugation and stored at -20°C before analysis. Serum obtained were assayed for total antioxidant activities, superoxide dismutase, glutathione peroxidase and catalase activities. Determination of serum total antioxidant capacity activities was carried out according to (Koracovic et al., 2001); Its principle is based on a standardised solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, leading to the formation of hydroxyl radicals. These reactive oxygen species degrade benzoate, resulting in the release of TBARS (Winterbourn, 1979; Gutteridge et al., 1990; Yamazaki and Piette 1990). Antioxidants from the added sample of fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development defined as the Antioxidant activity.

Superoxide dismutase (SOD) activity is estimated by the method of Marklund and Marklund (1974) adopted as follows by Soon and Tan (2002). It involves 2.1 ml of 50 mM buffer, 0.02 ml of enzyme source and 0.86 ml of distilled water. The reaction is initiated with 0.02 ml of 10 mM pyrogallol and change in absorbance monitored at 420 nm. One unit of SOD is defined as that amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50 % in standard assay system of 3 ml. The specific activity is expressed as units/min/mg protein.

Glutathione peroxidase activity is estimated as described by Rotruck et al. (1973) and Ellman (1959). Briefly, to 0.5 ml 0.4 M buffer pH 7.0), 0.2 ml enzyme source, 0.2 ml 2 mM GSH, 0.1 ml 0.2 mM H₂O₂ added and incubated at room temperature for 10 min along with a control tube containing all reagents except enzyme source. The reaction arrested by adding 0.5 ml of 10 % TCA, centrifuged at 4000 rpm for 5 min and the GSH content in 0.5 ml of supernatant was estimated. The activity expressed as µg of GSH consumed/min/mg protein.

Catalase activity is estimated by Beers and Sizer (1952) method. The assay system contains 1.9 ml 0.05 M buffer pH 7.0 and 1.0 ml 0.059 M H₂O₂. The reaction is initiated by addition of 0.1 ml enzyme source. The decrease in absorbance is monitored at 1 min interval for 5 min at 240 nm and activity is expressed as nmoles of H₂O₂ decomposed/ min/mg protein. Serum lipid

peroxidation was determined using thiobarbituric acid assay according to (Ohkawa et al., 1979)

STATISTICAL ANALYSIS

Data obtained in this study was subjected to ANOVA to detect significant effects with a confidence level of 95%, means were separated using Duncan multiple range test.

RESULT AND DISCUSSIONS

TOTAL ANTIOXIDANT ACTIVITY

Total antioxidant activity of laying pullets administered African Mistletoe leaf meal for 16 weeks is shown in **Figure 1**. At 8 weeks, Total antioxidant activity of laying pullets significantly ($P < 0.05$) increased with mistletoe inclusion. This shows that mistletoe endogenous antioxidant had enhance effect on laying pullets' total antioxidant activity in a dose dependent manner. This is in line with the claims of Ogechukwu et al. (2012) that antioxidation is one of the mechanism of action of mistletoe. This could be by stimulating the animal's enzymatic antioxidant system (GPx, SOD, Catalase) or non-enzymatic antioxidants (mistletoe alkali) present in mistletoe. However, at 16 weeks, serum total antioxidant activity of pullets were not significantly ($P > 0.05$) affected by dietary mistletoe leaf meal supplementation. This suggest that mistletoe could enhance total antioxidant activity of pullets at early lay. Although apparently higher total antioxidant activity were obtained in mistletoe fed groups at 16weeks of lay.

At 16 weeks, serum total antioxidant activity, superoxide dismutase and lipid peroxidase activity of pullets were not significantly ($P > 0.05$) affected by dietary mistletoe leaf meal supplementation. However, apparently higher total antioxidant activity and lower lipid peroxidation values were obtained in mistletoe fed groups. The trend of result show that total antioxidant activity of the pullets increased across the treatments

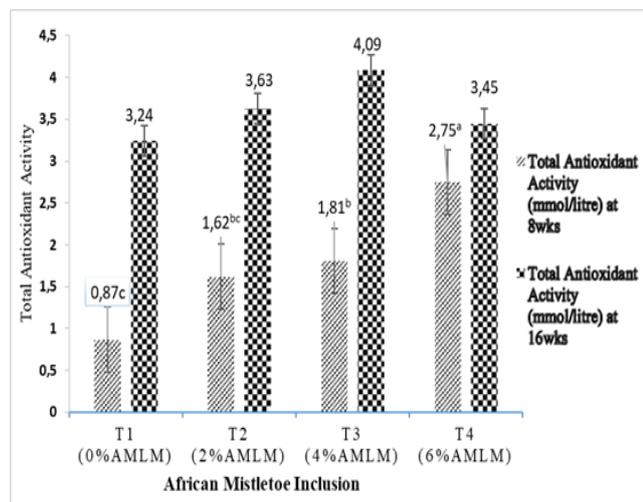


Figure 1. Total antioxidant activity of laying pullet fed mistletoe leaf meal supplemented diets (Actividad antioxidante total de las dietas suplementadas con harina de hoja de muérdago) abc: means with the same legend with different superscripts are significantly ($P < 0.05$) different.

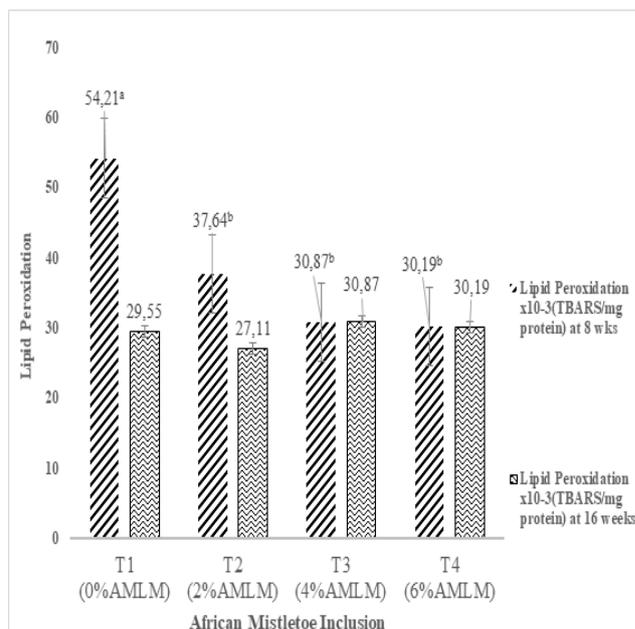


Figure 2. Serum lipid peroxidation of laying pullet fed mistletoe leaf meal supplemented diets (Peroxidación lipídica en suero de pollos alimentados con hojas de muérdago). abc: means with the same legend with different superscripts are significantly ($P < 0.05$) different.

from 8 to 16 weeks of lay. This suggest pullets possess higher protection from oxidation or higher total antioxidants as laying progresses. This may be due to higher requirement for steriodogenesis for maintainace of egg formation and/or presence of serum biomolecules that possess antioxidant properties.

LIPID PEROXIDATION

Serum Lipid peroxidation of laying pullets administered African Mistletoe leaf meal for 16 weeks is shown in **Figure 2**. Lipid peroxidation in laying pullets were significantly ($P < 0.05$) lowered by mistletoe supplementation in a dose dependent manner at 8weeks of lay. This could be attributed to the antioxidant status of the birds as influenced by the treatment administered. There is an inverse relationship between lipid peroxidation and antioxidant, increase in the formal leads to oxidative stress while increase in the later protects against free radical and peroxides. Mistletoe leaf meal reduce serum lipid oxidation in laying pullets at 30.56%, 43.05 and 44.31% at 1%,2% and 3% supplementation, respectively. This is in line with the report of Ogechukwu et al. (2012) that antioxidants from dietary sources also play important role in the control of oxidative stress, thus limiting cellular damage. Also mistletoe alkali (a component of mistletoe) acts as inhibitor of lipid peroxidation in rats (Shi et al., 2006).

However, at 16 weeks of lay lipid peroxidation of pullets were not significantly ($P > 0.05$) affected by dietary mistletoe leaf meal supplementation. This is similar to the trend obtained for total antioxidant activity, which implies mistletoe may not influence free radical accumulation at 16 weeks of lay. This may be due to higher total antioxidant demand with increase in egg production, which lowers free radical accumulation. The trend of

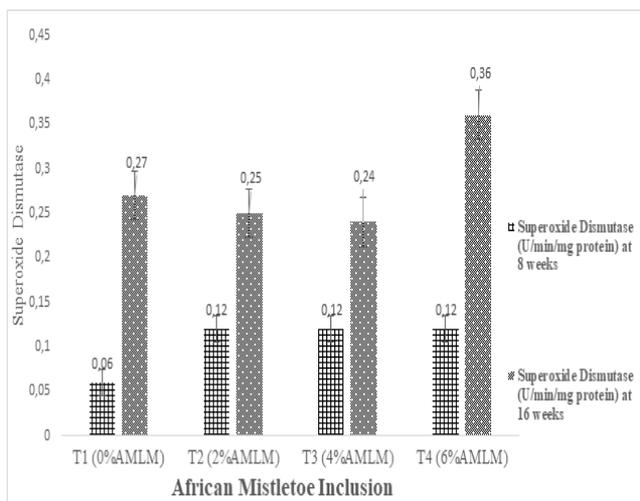


Figure 3. Serum Superoxide dismutase of laying pullet fed mistletoe leaf meal supplemented diets (Superóxido dismutasa de pollos alimentados con hojas de muérdago) abc: means with the same legend with different superscripts are significantly ($P < 0.05$) different.

result shows that pullets fed 4% and 6% AMLM supplementation recorded similar lipid peroxidation values at 8 and 16 weeks of feeding. However, birds on control and 2 % AMLM supplementation recorded lower values at 16 weeks. This implies that effective free radical scavenging ability of AMLM in laying pullets could be limited to shorter period of administration.

ANTIOXIDANT ENZYMES

SUPEROXIDE DISMUTASE

Superoxide dismutase activity of pullets fed AMLM is shown in **Figure 3**. Superoxide dismutase which is a first line defense against free radicals were not significantly ($P > 0.05$) affected by mistletoe supplementation throughout the study. Although apparent increase were observed in mistletoe treated groups compared to control. Similar to result of total antioxidant activity, superoxide dismutase activity apparently increased across the treatment at 16 weeks compared to values obtained at 8 weeks. This result is in agreement

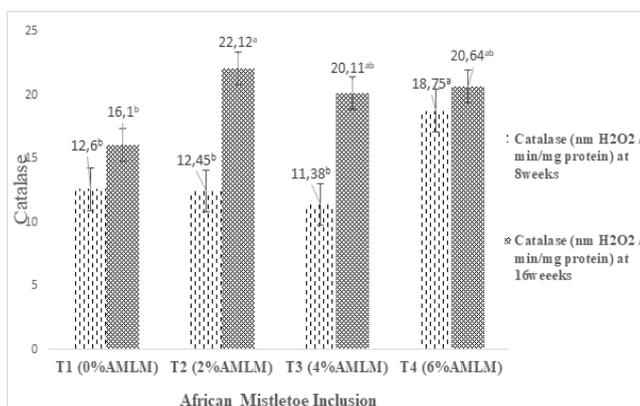


Figure 4. Serum Catalase of laying pullet fed mistletoe leaf meal supplemented diets (Catalasa sérica de las dietas suplementadas con harina de hoja de muérdago) abc: means with the same legend with different superscripts are significantly ($P < 0.05$) different.

with Mujahid et al., (2005) who reported no increase in superoxide production in heat stress-treated skeletal muscle mitochondria of laying chickens.

CATALASE

Serum Catalase activity of laying pullets is shown in **Figure 4**. At 8 weeks of feeding, serum catalase activity of birds fed 6% were statistically higher than other treatment. Reports that catalase and glutathione peroxidase are involved in the mitigation of hydrogen peroxide accumulation (Jimoh 2016) could account for this result. Contrariwise, serum catalase activities in pullets fed 2% mistletoe supplementation for 16 weeks were significantly ($P < 0.05$) higher than the control. While birds fed 2 and 3% AMLM had statistically similar values as control. Although, catalase activity increased across the treatments at 16 weeks, birds fed mistletoe had apparently higher values than the control. This is attributable to the consumption of AMLM, which could have stimulated endogenous production of catalase over the 16 weeks fed trial.

GLUTATHIONE PEROXIDASE

Serum glutathione peroxidase activity of laying pullets is shown in **Figure 5**. At 8 weeks, pullets fed 6% African mistletoe had significantly ($P < 0.05$) higher glutathione peroxidase activity than pullets on control diet. The result indicates that 6% mistletoe supplementation significantly ($P < 0.05$) enhanced hydrogen peroxide scavenging in laying pullets (via catalase and glutathione peroxidase activity). This is similar to report that indicated that mistletoe alkali not only scavenged OH directly but also inhibited OH generation in rats and mistletoe alkali could be a potential herbal medicine for improving GPX and SOD activity (Shi et al., 2006). At 16 weeks of feeding, serum glutathione peroxidase activity in birds fed mistletoe inclusive diets were significantly ($P < 0.05$) higher than birds on control. This is attributable to the consumption of AMLM, which could have stimulated endogenous production of glutathione peroxidase over the 16 weeks fed trial. This is in line with claims of Hashemipour et al., (2013) that active substances in thymol and carvacrol may improve the antioxidative status of broilers

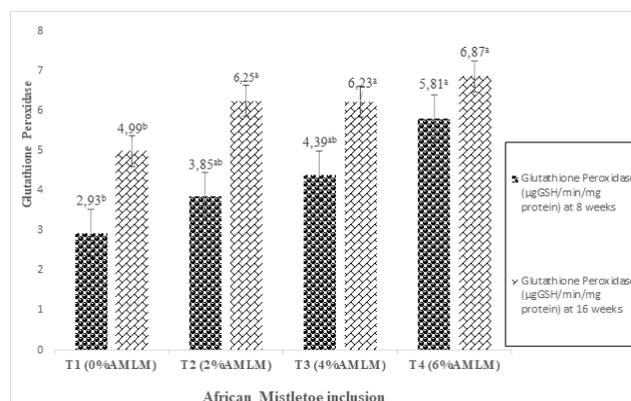


Figure 5. Serum glutathione peroxidase of laying pullet fed mistletoe leaf meal supplemented diets (Suero glutatión peroxidasa de la puesta de pollo alimentado con dietas de hoja de muérdago suplementado). abc: means with the same legend with different superscripts are significantly ($P < 0.05$) different.

due to the antioxidant property of thymol and carvacrol by elevating the activity of antioxidant enzymes (Glutathione peroxidase and superoxide dismutase). Lin et al. (2003) reported that the intake of herbs in chickens results in an increase in serum antioxidant enzyme activities and a decrease in MDA level.

Total antioxidant activity and all antioxidant enzymes of laying pullets increased, as laying cycle progresses, while lipid peroxidation reduced. This is evident in values obtained at 8 weeks and 16 weeks of lay in this study. This could signify that laying pullets have better antioxidative stability at 16 weeks of lay compared than pullets at 8 weeks of lay.

Modulation of the immune system and optimizing oxidative processes in humans and animals with the aid of natural products represents a field of drug development-based research witnessing unprecedented upsurge in recent times (Nworu, 2007). Immune system is intricately interwoven with oxidative processes in the body. High oxidative stress usually breaks down immune system, precipitates radicals as well as severe diseases and this must be prevented (Ogechukwu et al., 2012). Even though, the body has developed a variety of ways to deal with damaging free radicals, antioxidants from dietary sources also play important role in their control, thus limiting cellular damage (Ogechukwu et al., 2012). The result obtained in this study revealed that dietary mistletoe supplement stimulates antioxidant activity and scavenge peroxide formation up to 8 weeks of lay and laying pullets have better antioxidant defense at 16 weeks of lay.

CONCLUSION

It can be concluded from this work that mistletoe inclusion in laying pullets diet enhance antioxidant profile and inhibits oxidative stress. However, more emphasis should be paid to management of oxidative stress at early lay, because birds have higher antioxidant defense as laying progresses.

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