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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Effects of dietary natural antioxidant supplementation on broiler chicken and Muscovy duck meat quality

Margherita Marzoni[1], Riccardo Chiarini[2], Annelisse Castillo[3], Isabella Romboli[1], Michele De Marco[4], Achille Schiavone[4]*

[1] Dipartimento di Scienze Veterinarie, Universita degli Studi di Pisa, V.le delle Piagge 2, 56124 Pisa, Italy

[2] Freelance professional, Via Gioberti 17, 56035 Lari (PI), Italy

[3] Freelance professional, Apicastello honey farm, Via Pisana-Livornese 37, 57023, Cecina (PI), Italy

[4] Dipartimento di Scienze Veterinarie, Universita degli Studi di Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Torino, Italy

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A total of 150 one-day-old male broiler chicks (Ross 308) and 120 one-day-old female Muscovy ducklings were distributed over 15 and 12 pens, respectively. All birds received the same diet during the first period of life. Throughout the second period (36-56 days for broiler chickens and 43-69 days for Muscovy ducks) different source plant extracts were supplemented to the basal diet for each species; dietary treatments were assigned to three pens each. In the chicken (CK) trial the following dry extracts were tested: tomato (*Solanum lycopersicum)* skin (200 mg lycopene kg^{-1} feed; CK-L200 group), orange (*Citrus aurantium*) peel (200 mg hesperidin kg^{-1} feed; CK-O200 group), and green tea (*Camellia sinensis*) leaves (200 mg catechins kg^{-1} feed; CK-T200 group). For the Muscovy duck (DK) trial the tested extracts were produced from rosemary (*Rosmarinus officinalis)* leaves (200 mg carnosic acid kg^{-1} feed; DK-R200 group) and orange (*Citrus aurantium*) peel (200 mg hesperidin kg^{-1} feed; DK-O200 group). The effects in both species were compared with those for the unsupplemented diet (CK-C and DK-C) and the diet supplemented with 200 mg of alpha-tocopheryl acetate (CK-E200 and DK-E200). At the end of each trial three birds per pen were slaughtered. Growth performance, pH and meat proximate composition in both species were not affected by dietary treatments. The TBARS value of chicken leg meat from the unsupplemented group was 3.86, while on average in CK-E200, CK-L200 and CK-O200 it was by 60, 55 and 63% lower ($P<0.05$), whereas in CK-T200 it was by 25% higher ($P<0.05$). Dietary treatments did not exert any antioxidant effects on chicken breast meat. The TBARS value of duck breast meat and leg meat from the control was 1.39 and 4.51,

*Corresponding author: achille.schiavone@unito.it

respectively, while on average in the DK-E200, DK-O200 and DK-R200 groups it was by 82 and 71%, 33 and 46%, and 66 and 47% lower (P<0.05), respectively. The magnitude of the antioxidant action of vegetable dry extract in this trial was lower than that of alpha-tocopheryl acetate.

KEY WORDS: broiler chicken / meat quality / muscovy duck / plant antioxidants / TBARS value / vitamin E

Lipid stability of animal products during storage depends on the pro-oxidant and antioxidant content, fat content, the fatty acid profiles of fat and the degree of processing as well as storage conditions of products. It is well known that the quality of dietary lipids and dietary supplementation with supranutritional amounts of antioxidants, such as α-tocopheryl acetate (α-TA), significantly improves quality of poultry products (meat and eggs) through the improvement of lipid stability during storage [Bou *et al*. 2009].

Several studies have been conducted in vivo in order to test natural compounds able to improve lipid stability in meat. It has been demonstrated that dietary supplementation with both synthetic and natural antioxidants such as vitamin E [Avanzo *et al*. 2001, Schiavone *et al*. 2010a], ascorbic acid [Florou-Paneri *et al*. 2006], selenium [Avanzo *et al*. 2001], oat polyphenols [Lopez-Bote *et al*. 1998a], rosemary, sage and oregano extracts [Lopez-Bote *et al*. 1998, Florou-Paneri *et al*. 2006] and Silybum marianum fruit extract [Schiavone *et al*. 2007] may improve antioxidant defences and meat shelf life. These researches were widely reviewed by Bou *et al*. [2009].

Poultry are exposed to a multitude of long- and short-term stressors (e.g. heat stress, immune challenges, catching, transport), which may alter their internal homeostasis and oxidant/antioxidant balance, leading to oxidative stress, potentially having detrimental effects on meat shelf life [Young *et al*. 2003].

Thus, the aim of this study was to evaluate the effects of dietary supplementation of selected vegetable dry extracts on performance and meat quality in broiler chickens and Muscovy ducks.

Material and methods

An experimental study was designed involving male broiler chickens (ROSS 308) and female Muscovy ducks selected for meat production (S.A. Grimaud Frčre, France). The study was carried out on an environmentally controlled farm at the Avian Experimental Station of the Department of Veterinary Science, the University of Pisa (Italy). Housing conditions were in accordance with the "European Directive for the protection of vertebrate animals used for experimental and other specific purposes" [86/609/EU].

Animals and diets

A total of 150 one-day-old male broiler chicks and 120 one-day-old female ducklings were distributed over 15 and 12 pens (1.8 m×1.4 m), respectively. The stocking density

in each pen was 4 birds/m^2. Feed and water were provided *ad libitum*. Individual body weight (BW) and feed intake per pen were recorded weekly to calculate the feed conversion ratio (FCR). Their health status was controlled daily. All birds received the same diet (separately for chickens and ducks) during the first period of life (1-35 days and 1-42 days for chickens and Muscovy ducks, respectively) (Tab. 1). Throughout the second period (36-56 days for broiler chickens and 43-69 days for Muscovy ducks) different dietary treatments were randomly assigned to three pens each. The end of the trial was at 56 d and 69 d of age for chickens and ducks, respectively, when some birds were slaughtered. The diets were formulated to meet or exceed the requirements indicated by the NRC [1994] both for chickens and ducks. The composition of the basal diets for both species is shown in table 1. In the chicken (CK) trial, the experimental diets were designed as follow: CK-C: the basal diet; CK-E200: the basal diet supplemented with 200 mg kg^{-1} alpha-tocopheryl acetate (α-TA); CK-L200: the basal diet supplemented with dry extract from tomato (*Solanum lycopersicum)* skin in order to provide 200 mg lycopene kg^{-1} feed; CK-O200: the basal diet supplemented with dry extract from orange (*Citrus aurantium*) peel to provide 200 mg hesperidin kg^{-1} feed; CK-T200: the basal diet supplemented with dry extract from green tea (*Camellia sinensis*) leaves to provide 200 mg catechins kg^{-1} feed. In the Muscovy duck (DK) trial the diets were designed as follow: DK-C: the basal diet; DK-E200: the basal diet supplemented with 200 mg kg^{-1} alpha-tocopheryl acetate (α-TA); DK-R200: the basal diet supplemented with dry extract from rosemary (*Rosmarinus officinalis)* leaves to provide 200 mg

Table 1. Composition of diets

Item	Broiler chicken		Muscovy duck	
	1-35d	36-56d	1d-42d	43d-69d
Ingredients (g/kg)				
corn	570	660	598	600
soybean meal (48% cp)	358	253	335	314
soy oil	30	40	20	20
alfalfa meal	-	-	-	20
dicalcium phosphate	20	20	20	20
limestone	10	17	17	17
sodium chloride	2	2	2	2
DL-methionine	4	2	2	1
L-lysine	1	1	1	1
vitamin and mineral premix[1]	5	5	5	5
Chemical composition (g/kg)				
dry matter	897	905	877	878
crude protein	196	169	225	200
ether extract	56	60	34	37
crude fibre	26	29	23	32
ash	69	52	67	65
Metabolizable energy (mj/kg)	12.5	13.0	12.3	12.6

[1]Provided per kg of diet: retinol 3 mg; cholecalciferol, 45 mg; DL-α-tocopheryl acetate 30 mg; thiamine 1.5 mg; riboflavin 3 mg; pyridoxine 1.5 mg; cobalamin 0.015 mg; pantothenic acid 8.0 mg; niacin 25 mg; choline chloride 500 mg; Fe ($FeSO_4 \cdot 7H_2O$) 30 mg; Cu ($CuSO_4 \cdot 5H_2O$) 1.5 mg; Mn ($MnSO_4 \cdot H_2O$) 80 mg; Zn ($ZnSO_4 \cdot 7H_2O$) 30.0 mg; I (KI) 1.4 mg.

carnosic acid kg^{-1} feed; DK-O200: the basal diet supplemented with dry extract from orange (*Citrus aurantium*) peel to provide 200 mg hesperidin kg^{-1} feed. All vegetable dry extracts were purchased at Sochim International S.p.A. [20154 Milano – Italy]. The chemical composition of the diets was evaluated according to the Association of Official Analytical Chemists (AOAC) procedures [2004].

Slaughtering procedures

At slaughtering age (56 d and 69 d for chickens and ducks, respectively) three birds per pen were sacrificed by electrical stunning followed by decapitation, after a 8-hour fasting period. Eviscerated and plucked carcasses were weighed after removal of the head, neck, feet and abdominal fat to obtain ready-to-cook carcasses (RCC). Carcasses were stored in a cool chamber, at 0 to 4°C, until the next day when carcass yield and pH [Hanna Instruments 8417 pH-meter supplied with a Hamilton Biotrode electrode] of *m. pectoralis major and m. iliotibialis* were determined.

Proximate and TBARS analysis

At carcass dissection, breasts and thighs were vacuum packaged and immediately frozen (-20°C) pending analysis. The AOAC methods [2004] were used to assess moisture, ash, protein and ether extract and the results were expressed as percentages on a fresh matter basis. Susceptibility to lipid oxidation, based on thiobarbituric acid reactive substances (TBARS), was evaluated according to the Iron-induced TBARS procedure, as described by Huang and Miller [1993]. Briefly, 3 g of minced breast or thigh meat were homogenized in 57 ml of 1.15% KCl chilled solution; 30 ml of the homogenate were incubated at 37°C in a shaking water bath with 8.34 mg $FeSO_4 \cdot 7H_2O$ (final concentration 1 mM Fe^{+3}) as the oxidative agent. Iron-induced TBARS assay was performed at 0, 30 and 60 minutes of incubation and the absorbance was read at 532 nm. Liquid malondialdehyde (MDA) (Aldrich Chemical Co Ltd, Dorset England) was used as the standard to determine the linear standard response and recovery. TBARS values were calculated by multiplying absorbance by a constant coefficient K (23.58), combining standard response, recovery (93.4%), molecular weight of the MDA and sample weight. TBARS values are expressed as mg MDA kg^{-1} fresh meat.

Statistical analysis

Each pen was considered the experimental unit for growth performance traits. The individual animal was the experimental unit for slaughter traits, pH, TBARS and proximate meat composition. Before testing for group differences, normality of data distribution and the homogeneity of variance were assessed using the Shapiro-Wilk test and the Levene test, respectively. One-way ANOVA was applied to data considering the dietary treatment as fixed effect. The statistical analyses were performed using the SPSS software package [2008]. The differences were tested using Tukey's post-hoc test. Significance was accepted for $P<0.05$ and results are presented as mean and pooled standard error of the mean (SEM).

Results and discussion

Beneficial features of plant extracts are increasingly used in the composition of feed for livestock use. These mixed plant products contain active substances in different amounts and their activity varies to a great extent between plant species, depending also on the harvest period, technology of drying and extraction processes. Several studies reported positive effects of medicinal plants related to their bioactive compounds such as carvacrol that affect feed intake, secretion of gastrointestinal fluids and improve digestion and absorption processes and subsequent weight gain in broiler chickens [Khaligh *et al*. 2011]. In the present study the α-TA or vegetable extract supplementation did not exert any effects on growth performance or slaughter yields for either broiler chickens or Muscovy ducks. The effect of vegetable extract supplementation in optimizing the FCR is controversial and it depends on the amount, source and period of administration [Hernandez *et al*. 2004].

All data resulted normally distributed and variance was homegeneus.

The TBARS is frequently used to evaluate lipid oxidation. In our trial α-TA supplementation resulted in an improved resistance against oxidation in both chicken and Muscovy ducks (Tab. 2 and 3). Numerous studies reported the protective effect of dietary α-TA against lipid oxidation both *in vivo* and in the muscle, so as to prolong the shelf life of meat. Among poultry species the majority of studies have been performed on the chicken, with the turkey ranking second. In particular, these studies assessed the effect of dietary tocopherols supplementation on lipid stability of chicken meat [Bou *et al.* 2009]. Additionally, the protective effect of α-TA supplemented diets has been shown *in vivo* in the chicken by the evaluation of the improved resistance of erythrocytes against haemolysis and lipid peroxidation [Soto-Salanova and Sell 1996, Schiavone *et al*. 2010b]. Lipid peroxidation in duck meat has been studied to a lesser extent. However, some authors confirm the protective effect of α-TA against lipid oxidation even in meat from ducks fed a supranutritional amount of α-TA [Russell *et al*. 2004, Schiavone *et al*. 2010a].

In the present study tomato skin dry extract supplementation exerted an antioxidant effect in chicken thigh meat (Tab. 2), while no protective effect was found in breast meat. The magnitude of the antioxidant effect was similar to that observed for α-TA supplemented groups. Conflicting effects of lycopene on poultry meat oxidative stability were found. No effect of lipid stability in broiler chicken liver or meat was found by Leal *et al*. [1999] in birds fed 25 mg lycopene kg^{-1} body weight day^{-1}, and by Ševčíková *et al*. [2008] in birds fed 50-100 mg lycopene kg^{-1} feed. An antioxidant effect of lycopene was demonstrated in raw meat of Japanese quail fed 50-100 mg lycopene kg^{-1} feed [Sahin *et al*. 2008]. In Japanese quail Botsoglou *et al*. [2004] found an antioxidant effect in raw and cooked meat when birds were fed 50 g kg^{-1} feed dried tomato pulp, while a pro-oxidant effect was found at the dried tomato pulp inclusion at 100 g kg^{-1} feed. Adverse effects (weight decrease and degenerative lesions in the spleen and bursa of Fabricious; reduced concentrations of serum protein, albumin, alpha and

Table 2. Effects of dietary treatments for a 3-week period on growth performance, slaughter and meat traits of broiler chickens (n=15 for growth performance; n=45 for slaughter and meat traits)

Item	CK-C[1]	CK-E200[2]	CK-L200[3]	CK-O200[4]	CK-T200[5]	SEM
BW[6] 35d (g)	1617	1615	1618	1621	1617	5.503
BW 56d (g)	3435	3476	4322	3471	3424	20.555
FCR[7] (35-56d)	2.36	2.32	2.27	2.39	2.37	0.019
FBW (g)[8]	3432	3429	3442	3475	3449	14.282
RCC (g)[9]	2487	2491	2475	2488	2494	5.542
%FBW	72.5	72.7	71.9	71.6	72.3	0.288
Breast (g)	727	731	725	721	726	1.856
%FBW	21.2	21.3	21.1	20.8	21.0	0.097
Thighs (g)	850	857	844	849	850	2.955
%FBW	24.8	25.0	24.5	24.4	24.7	0.093
Breast meat						
Moisture (%)	73.1	72.9	72.6	73.0	73.1	0.020
Protein (%)	24.0	24.0	24.4	24.5	24.5	0.015
Lipid (%)	0.9	1.0	0.8	0.9	0.9	0.021
Ash (%)	1.2	1.3	1.2	1.3	1.2	0.055
pH_{24h}	5.8	5.8	5.8	5.8	5.7	0.005
TBARS 0 min	0.1^{b}	0.12^{b}	0.36^{a}	0.15^{b}	0.16^{b}	0.022
TBARS 30 min	0.45	0.45	0.49	0.55	0.60	0.022
TBARS 60 min	0.81^{bc}	0.67^{c}	1.05^{a}	0.95^{ab}	0.73^{c}	0.037
TBARS average	$0,46^{c}$	$0,41^{c}$	$0,64^{a}$	$0,55^{ab}$	$0,50^{bc}$	0.019
Thigh meat						
Moisture (%)	74.04	74.28	74.31	74.42	74.35	0.059
Protein (%)	18.41	18.40	18.30	18.40	18.35	0.049
Lipid (%)	1.46	1.42	1.48	1.40	1.42	0.021
Ash (%)	1.34	1.32	1.25	1.39	1.38	0.025
pH_{24h}	5.90	5.91	5.90	5.90	5.90	0.004
TBARS 0 min	0.26	0.26	0.19	0.20	0.26	0.010
TBARS 30 min	4.35^{A}	1.36^{B}	1.49^{B}	1.36^{B}	4.66^{A}	0.350
TBARS 60 min	6.98^{AB}	2.99^{BC}	3.51^{BC}	2.78^{C}	9.55^{A}	0.616
TBARS average	3.86^{B}	1.54^{CD}	1.73^{C}	1.45^{D}	4.82^{A}	0.320

[aA]...Means within rows bearing the same superscript differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

[1]CK-C – basal diet; [2]CK-E200 – basal diet supplemented with 200 mg kg^{-1} alpha-tocopheryl acetate (α-TA); [3]CK-L200 – basal diet supplemented with dry extract from tomato (*Solanum lycopersicum)* skin to provide 200 mg kg^{-1} lycopene; [4]CK-O200 – basal diet supplemented with dry extract from orange (*Citrus aurantium*) peel to provide 200 mg kg^{-1} hesperidin; [5]CK-T200 – basal diet supplemented with dry extract from green tea (*Camellia sinensis*) leaves to provide 200 mg kg^{-1} catechins. [6]BW – body weight; [7]FCR – feed conversion ratio; [8]FBW – final body weight of slaughtered birds; [9]RCC – ready to cook carcass; TBARS – thiobarbituric acid reactive substances expressed as mg malondialdehyde (MDA) kg^{-1} meat.

Table 3. Effects of dietary treatments for a 3-week period on growth performance, slaughter and meat traits of Muscovy ducks (n=12 for growth performance; n=36 for slaughter and meat traits)

Item		DK-C[1]	DK-E200[2]	DK-O200[3]	DK-R200[4]	SEM
BW[5] 42d (g)		1822	1833	1837	1838	12.345
BW 69d (g)		2563	2566	2567	2581	18.655
FCR[6] (43-69d)		4.05	3.89	4.01	3.98	0.035
FBW[7] d69 (g)		2588	2572	2557	2559	12.901
RCC[8] (g)		1641	1632	1642	1637	3.752
	%FBW	63.4	63.5	64.2	64.0	0.271
Breast (g)		332	321	321	331	2.371
	%FBW	12.9	12.5	12.6	12.9	0.093
Thighs (g)		451	457	452	456	3.323
	%FBW	17.4	17.8	17.6	17.8	0.120
Breast meat						
Moisture (%)		76.16	76.44	76.41	76.12	0.095
Protein (%)		21.73	21.96	21.85	21.78	0.081
Lipid (%)		1.52	1.52	1.51	1.50	0.009
Ash (%)		1.29	1.28	1.29	1.29	0.014
pH_{24h}		5.75	5.76	5.75	5.76	0.003
TBARS 0 min		0.26^{a}	0.19^{b}	0.24^{ab}	0.21^{ab}	0.010
TBARS 30 min		1.54^{A}	0.25^{D}	1.23^{B}	0.54^{C}	0.137
TBARS 60 min		2.37^{A}	0.29^{D}	1.33^{B}	0.67^{C}	0.206
TBARS average		1.39^{A}	0.24^{D}	0.93^{B}	0.47^{C}	0.148
Thigh meat						
Moisture (%)		74.58	74.57	74.32	74.83	0.125
Protein (%)		19.24	19.22	19.14	19.32	0.048
Lipid (%)		3.19	3.23	3.15	3.27	0.047
Ash (%)		1.24	1.23	1.24	1.23	0.012
pH_{24h}		5.85	5.85	5.84	5.84	0.007
TBARS 0 min		0.30	0.28	0.30	0.32	0.008
TBARS 30 min		5.62^{A}	1.26^{C}	2.45^{B}	2.61^{B}	0.378
TBARS 60 min		7.61^{A}	2.37^{C}	4.51^{B}	4.28^{B}	0.485
TBARS average		4.51^{A}	1.30^{C}	2.42^{B}	2.40^{B}	0.288

[aA...] Means within rows bearing the same superscript differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

[1]DK-C – basal diet; [2]DK-E200 – basal diet supplemented with 200 mg kg^{-1} alpha-tocopheryl acetate (α-TA); [3]DK-O200 – basal diet supplemented with dry extract from orange (*Citrus aurantium*) peel to provide 200 mg kg^{-1} hesperidin; [4]DK-R200 – basal diet supplemented with dry extract from rosemary (*Rosmarinus officinalis)* leaves to provide 200 mg kg^{-1} carnosic acid; [5]BW – body weight; [6]FCR – feed conversion ratio; [7]FBW – final body weight of slaughtered birds; [8]RCC – ready to cook carcass; TBARS – thiobarbituric acid reactive substances expressed as mg malonaldehyde (MDA) kg^{-1} meat.

gamma globulin) were observed when lycopene was supplemented at the high dose of 500 mg kg^{-1} diet [Pozzo *et al*. 2013].

To our knowledge no other studies have been performed to assess the protective effect of dietary orange peel extract against meat lipid oxidation in poultry. In this study, an antioxidant effect of orange peel extract was found in both chickens (Tab. 2) and Muscovy ducks (Tab. 3). Orange peel extract contains hesperidin, a bioflavonoid with antioxidant activity both *in vitro* [Suarez *et al*. 1999] and *in vivo* [Miyake *et al*. 1998]. In broiler chicken nutrition citrus pulp has been used as a feed ingredient. It was observed that this ingredient reduced growth performance when included at 3% [Ebrahimi *et al*. 2013] or 5 and 10% [Mourao *et al*. 2008] of diet; however, inclusion of 1.5% citrus pulp in the diet did not affect growth performance [Ebrahimi *et al*. 2013].

In this trial, the green tea dry extract supplementation did not yield an antioxidant effect in chicken breast and thigh (Tab. 2). The antioxidant effect of tea catechins in poultry meat patties has been proven in many studies, in which catechins were added directly to meat as dry powder [Jeong *et al*. 2009, Tang *et al*. 2001] or by infusion [Rababah *et al*. 2010]. However, to our knowledge there have been no *in vivo* studies on poultry.

In the current study rosemary supplementation induced an antioxidant effect in Muscovy duck meat (Tab. 3). This result is in accordance with previous findings in broiler chickens fed diets supplemented with rosemary extracts or oleoresins. Lopez-Bote *et al*. [1998] found an antioxidant effect by testing the dose of 500 mg carnosic acid kg^{-1} diet.

Summarizing, TBARS of chicken leg meat from the unsupplemented group was 3.86, while on average in CK-E200, CK-L200 and CK-O200 it was by 60, 55 and 63% lower ($P<0.05$), whereas in CK-T200 it was by 25% higher ($P<0.05$). Dietary treatments did not exert any antioxidant effects in chicken breast meat. TBARS value of duck breast meat and leg meat from the control was 1.39 and 4.51, respectively. In the DK-E200, DK-O200 and DK-R200 groups it was by 82 and 71%, 33 and 46%, and 66 and 47% lower ($P<0.05$), respectively. The magnitude of the antioxidant action of vegetable dry extract in this trial was lower than that of α-TA. The fat and iron content of duck meat is higher in comparison with meat of other poultry species [Chartrin *et al*. 2006], such as chicken and turkey. It leads to a higher susceptibility of duck meat to peroxidation during storage. Therefore improving duck meat antioxidant capacity through α-TA or vegetable extract supplementation is an approach of great interest for meat conservation. This improvement could be particularly important for duck meat due to its higher fat content when compared to meat from other poultry species more commonly used in human nutrition. In the present study the dietary treatments reduced TBARS values in both thigh and breast in broiler chicken and Muscovy duck, except for chicken fed green tea dry extract supplemented diets. This effect could be related to the improvement of *post mortem* antioxidant defences. The increased *post mortem* oxidative stability could rely on an increased concentration of polyphenol compounds in tissues, which directly inhibit lipid peroxidation, but also spare other antioxidant molecules and

enzymes. The magnitude of the antioxidant action of vegetable dry extract in this trial is not comparable with that of α-TA; however, the improved lipid stability was provided even by natural compounds, mainly from tomato, orange and rosemary dry extracts.

In conclusion data of the present study suggest that poultry meat shelf life could be improved by supranutritional amounts of α-tocopheryl acetate or by supplementation of diets with vegetable dry extracts.

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