

Histopathology of broiler chickens fed diets supplemented with *Prosopis africana* (African mesquite) essential oil

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Abstract

This study was carried out to investigate the hepatic histopathology of broiler chickens fed diets supplemented with *Prosopis africana* (African mesquite) essential oil (PRSO). 300, 1-day old broiler chicks were randomly distributed into six treatments with 5 replicates consisting of 10 birds each. Birds in diet 1 (D1) was fed basal diet supplemented with 0 % PRSO, D2: basal diet with 1.0 g/kg⁻¹ oxytetracycline, D3, D4, D5 and D6 were fed basal diet supplemented with PRSO at 200 mg, 400 mg, 600 mg and 800 mg/kg⁻¹ respectively. The experiment lasted for 56 days and a completely randomized design was adopted. Phyto-constituents of PRSO revealed the presence of tannins (201.21 mg/g⁻¹), flavonoids (677.83 mg/g⁻¹), alkaloids (405.90 mg/g⁻¹), saponins (75.62 mg/g⁻¹), phenols (508.28 mg/g⁻¹), phytates (20.10 mg/g⁻¹) and oxalates (5.26 mg/g⁻¹). Hepatic histopathology showed that tissues from D1, D3, D4, D5 and D6 showed no signs of any damage; the central vein, blood portal vessels showed no congestion. Mild hepatocellular damage was recorded among liver of birds in D2. It can be concluded that *Prosopis africana* essential oil are among the key alternative solutions to antibiotics because they contain bioactive compounds that are safe, effective and performs multiple biological functions in the body of animals.

Keywords: *Prosopis africana* oil, phytochemicals, broilers, liver, histopathology.

Histopatologia de frangos de corte alimentados com dietas suplementadas com óleo essencial de *Prosopis africana* (mesquita africana)

Resumo

Este estudo foi realizado para investigar a histopatologia hepática de frangos de corte alimentados com dietas suplementadas com óleo essencial de *Prosopis africana* (mesquita africana) (PRSO). 300 pintos de corte de 1 dia de idade foram distribuídos aleatoriamente em seis tratamentos com 5 repetições de 10 aves cada. Aves na dieta 1 (D1) receberam dieta basal suplementada com 0 % de PRSO, D2: dieta basal com 1,0 g/kg⁻¹ de oxitetraciclina, D3, D4, D5 e D6 receberam dieta basal suplementada com PRSO a 200 mg, 400 mg, 600 mg e 800 mg/kg⁻¹, respectivamente. O experimento teve duração de 56 dias e foi adotado o delineamento inteiramente casualizado. Os fitoconstituintes do PRSO revelaram a presença de taninos (201,21 mg/g⁻¹), flavonoides (677,83 mg/g⁻¹), alcaloides (405,90 mg/g⁻¹), saponinas (75,62 mg/g⁻¹), fenóis (508,28 mg/g⁻¹), fitatos (20,10 mg/g⁻¹) e oxalatos (5,26 mg/g⁻¹). A histopatologia hepática mostrou que os tecidos de D1, D3, D4, D5 e D6 não apresentavam sinais de danos; a veia central, os vasos sanguíneos portais não apresentaram congestão. Danos hepatocelulares leves foram registrados entre fígados de aves em D2. Pode-se concluir que o óleo essencial de *Prosopis africana* está entre as principais soluções alternativas aos antibióticos porque contém compostos bioativos que são seguros, eficazes e desempenham múltiplas funções biológicas no corpo dos animais.

Palavras-chave: óleo de *Prosopis africana*, fitoquímicos, frangos de corte, fígado, histopatologia.

1. Introduction

Nowadays, there is an increasing awareness on the need to maintain good health via the use of natural products from plants because they are efficient, cheap, easily metabolized with minimum health hazards and do not pose

any environmental pollution (Alagbe, 2022; Wallace et al., 2011). Medicinal plants are also known as phytochemical feed additives are regarded as complex mixtures of bioactive components or phytochemicals found in varying amount depending on geographical location, stage of growth or harvesting, parts used (leaf, seeds, bark, buds, flowers and twig) as well as method of processing (Shittu; Alagbe, 2020; Simitzis, 2017).

Bioactive compounds or phytochemicals from plants includes: phenolics, flavonoids, terpenoids, carotenoids, saponins, alkaloids, flavours and fragrances. Plant extracts (essential oils) with these bioactive molecules are widely used in the food, pharmaceutical and cosmetic industries (Lewis et al., 2003; Alagbe, 2017). They are also capable of enhancing livestock productivity through the improvement of digestibility, nutrient absorption and elimination of pathogens residents in the animal gut (Denli et al., 2004). Medicinal plants had possessed antimicrobial (Agubosi et al., 2022; Nursoy et al., 2011), antioxidants (Adewale et al., 2021), antibacterial (Dhama et al., 2015), antiviral (Mehr et al., 2014), immune-modulatory (Krishan; Narang, 2014; Olorunmaiye et al., 2019), hepato-protective, antifungal and cytotoxic (Rostagno et al., 2011; Souza et al., 2015), antifungal, anti-proliferative, anti-helminthic, antiviral and anti-tumor (Souza et al., 2015), anti-cancer (Assis et al., 2017) and anti-protozoal, anti-proliferative, anticoagulant, anticonvulsant, antipsychotic (Brenes; Roura, 2010; Cho et al., 2014).

Essential oils (EOs) has shown positive effect in livestock by promoting growth, relieving the host animals from immune defense stress during critical situations, scavenging free radicals capable of causing diseases and increase the intestinal availability of essential nutrients for absorption, thereby helping animals to grow better within the framework of their genetic potential (Lee et al., 2003; Bozkurt et al., 2016). EOs have been demonstrated to work through the antioxidative mechanisms serving as free radical scavengers promoted by polyphenols, flavonoids and terpenoids which possess the ability to protect the cell organelles from damage caused by free radicals induced oxidative stress either by inhibiting the initiation or propagation of oxidative chain reactions (Sevim et al., 2017; Zeng et al., 2015). Studies have shown that the plants active principle agents in the herbs have a strong capability for scavenging superoxide radicals, hydrogen peroxide and nitric oxide from activated macrophages, reducing iron complex and inhibiting lipid peroxidation (Stef et al., 2009; Umashanker et al., 2011).

Prosopis africana (African mesquite) is a leguminous multipurpose tree that belongs to the family Fabaceae and subfamily *Mimosoideae* (Ogunshe et al., 2017). It is known as Iron wood in English, Kiriya in Hausa, Ubwa in Igbo and it consist of 14 species and widely grown in tropical, arid and semi arid regions of the world (Olorunmaiye et al., 2019). Essential oils can be extracted mostly from the seed which contains compounds with therapeutic properties (alkaloids, saponins, tannins, phenols, cardiac glycosides, flavonoids and terpenoids) (Olorunmaiye et al., 2019; Gberikon et al., 2015; Ugwoke et al., 2019).

Traditionally, the roots and stem bark extracts are used for the treatment and prevention of fever, skin diseases, diarrhea, dysentery, respiratory disorders, rheumatism, pneumonia and ulcer (Gberikon et al., 2015; Oguntoyinbo et al., 2017). *Prosopis africana* oil can also inhibit the growth of pathogenic microorganisms; *Staphylococcus* spp, *Bacillus* spp, *Escherichia coli*, *Enterobacter* spp, *Enterococcus* spp, *Pseudomonas aeruginosa*, *Shigella* spp, *Klebsiella* spp and *Proteus* spp (Alagbe, 2023; Oguntoyinbo et al., 2007; Gordon et al., 1973).

There is no justification for assuming that just because essential oils are natural, they must therefore be safe; certain phytochemicals or bioactive compounds can interfere with the body's system for metabolizing medications in the liver, which can have a chain reaction of negative effects. Therefore, this experiment was designed to investigate the histopathology of broiler chickens fed diets supplemented with *Prosopis africana* (African mesquite) essential oil.

2. Materials and Methods

2.1 Ethical approval, collection and processing of *Prosopis africana* oil (PRSO)

The study was carried out at the University of Abuja Teaching and Research Farm in the month of April - June, 2022 according to the guidelines of animal protocol agreed by the Research and Ethic committee (ANSJ/004) of the Department of Animal Science, University of Abuja, Gwagwalada, Nigeria.

Fresh seeds of *Prosopis africana* were purchased from a local market in Gwagwalada, authenticated at the Department of Biological Sciences, University of Abuja by a certified taxonomist where a Voucher specimen number PRO/ANS/004 was deposited at the herbarium of the same department. Air dried on a clean metallic plate for 11 days and grinded into powder using a blender thereafter poured into a well labeled zip log before it was transported to a laboratory for further laboratory examination.

Prosopis africana oil was extracted employing the steam-distillation method, which also calls for the use of a digital scale, a round bottom flask, distilled water, a heating mantle made of glass yarn, a measuring cylinder, and a separatory funnel. 250 mL of distilled water was added to a round-bottom flask along with 50 grams of ground *Prosopis* seed. The mixture was added to a glass yarn heating mantle, warmed to a temperature of 80 °C, and then the condenser was positioned above the flask with a round bottom. The combination is forcefully boiled for 15 min, the distillate is collected in a beaker until no more oil droplets flow over, 5 grams of sodium chloride are added, and the mixture is constantly agitated. To get *Prosopis africana* oil, the distillate was then put into the separatory funnel and evaporated in a steam bath.

2.2 Management of experimental animals and design

300, 1-day old broiler chicks of mixed sex (Arbo Acres) were purchased from a reputable commercial hatchery in Oyo State, Nigeria and randomly distributed into six treatments with 5 replicates consisting of 10 birds each. A galvanized battery cage measuring (100 cm × 75 cm × 50 cm) (length × depth × height) was equipped with automatic nipple drinkers and galvanized manual feeder in a semi open pens. Chicks were weighed on arrival to determine their average initial body weight and thereafter on weekly basis and given anti-stress (glucose + water) at the rate of 2 grams of glucose to 10 liters of water. Completely randomized experimental design was used in this study.

2.3 Experimental diets

Diets were compounded based on the nutrient requirements for broilers according to Olomu (2021) as presented in (Table 1). Experimental diet at the starter phase (1-28 days) and finisher phase (29-56 days) contains 23.06% crude protein (CP), 21.40% CP and metabolizable energy content (ME) of 2991.5 Kcal/kg⁻¹ and 3108.1 Kcal/kg⁻¹ dry matter respectively. All birds had unrestricted access to feed and water throughout the entire experimental period (8 weeks).

Experimental set-up is shown below:

Diet 1 (D1): Basal diet with no *Prosopis africana* oil (Negative control)

Diet 2 (D2): Basal diet + Oxytetracycline 1.00 g/kg⁻¹ (Positive control)

Diet 3 (D3): Basal diet + 200 mg/kg⁻¹ *Prosopis africana* oil (PRSO)

Diet 4 (D4): Basal diet + 400 mg/kg⁻¹ PRSO

Diet 5 (D5): Basal diet + 600 mg/kg⁻¹ PRSO

Diet 6 (D6): Basal diet + 800 mg/kg⁻¹ PRSO

2.4 Determination of phytochemical components of *Prosopis africana* oil

Phenols and total tannins in PSO were evaluated using *Folin-Ciocalteu* method outlined by Otles and Yacin (2012). Total flavonoids, alkaloids, phytates and oxalates were determined using the methods described by Harborne (1973); Odebiyi and Sofowora (1978). Saponins was quantified using the protocols used by Brunner (1984).

2.5 Histopathological evaluation

Liver specimens (1 cubic cm) were taken on the 56th day of the experiment and placed in a well-sealed leak proof labelled container containing 10% formalin. The 10% formalin solution prevents the tissues from degeneration. Thereafter, specimens were transported at room temperature to the laboratory for further examination. All tissues were stained with Hematoxylin and Eosin (H & E) stain and the histopathological changes were observed under a microscope.

Table 1. Ingredient composition of the experimental diets.

Materials	Starters mash (1-28 days)	Finishers mash (29-56 days)
Maize	52.55	57.55
Wheat offal	2.00	5.00
Soya bean meal	29.00	22.45
Groundnut cake	8.00	5.00
Fish meal (72%)	3.00	3.00
Limestone	1.50	2.00
Bone meal	3.00	4.00
Lysine	0.20	0.20
Methionine	0.20	0.20
**Premix	0.25	0.25
Salt	0.30	0.35
Total	100.0	100.0
Determined analysis (% DM)		
Crude protein	23.06	21.40
Crude fibre	4.00	5.30
Ether extract	4.03	4.47
Calcium	1.66	1.83
Phosphorus	0.78	0.99
Energy (Kcal/kg ⁻¹)	2991.5	3108.1

Note: *Premix supplied per kg diet: - vit A, 10,000 I.U; vit E, 5 mg; vit D3, 3000 I.U, vit K, 3 mg; vit B2, 5.5 mg; Niacin, 25 mg; vit B12, 16 mg; choline chloride, 120 mg; Mn, 5.2 mg; Zn, 25 mg; Cu, 2.6 g; folic acid, 2 mg; Fe, 5g; pantothenic acid, 10 mg; biotin, 30.5 g; antioxidant, 56 mg (starter's mash). **Premix supplied per kg diet: - vit A, 8,500 I.U; vit E, 10 mg; vit D3, 1500 I.U, vit K, 3.8 mg; vit B2, 10 mg; Niacin, 15 mg; vit B12, 10 mg; choline chloride, 250 mg; Mn, 5.0 mg; Zn, 56 mg; Cu, 1.6 g; folic acid, 2.8 mg; Fe, 5.1 g; pantothenic acid, 10 mg; biotin, 30.5 g; antioxidant, 56 mg (finisher's mash). Source: Autors, 2023.

3. Results and Discussion

3.1 Phytochemical constituents of *Prosopis africana* oil

Phytochemical constituents of *Prosopis africana* oil is summarized in (Table 2 and Figure 1). Flavonoids had the highest concentration (677.83 mg/g⁻¹) followed by phenols (508.28 mg/g⁻¹), alkaloids (405.90 mg/g⁻¹), tannins (201.21 mg/g⁻¹), saponins (72.62 mg/g⁻¹), phytates (20.10 mg/g⁻¹) and oxalates (5.26 mg/g⁻¹) respectively. All the phytochemicals have stated above have different medicinal and pharmacological importance. For instance, flavonoids therapeutically function as strong antimicrobial against pathogenic organisms (Alagbe, 2020), antioxidants against reactive oxygen species which are generated from free radicals (Oluwafemi et al., 2021).

Alkaloids have several pharmacological properties on human and livestock health including anti-inflammatory, analgesics, antifungal, antimalarial, anti-hypertensive, neuro-protective, anti-parasitic and anti-tumor (Xie et al., 2021; Roy, 2017). High concentrations of alkaloids in the body system can lead to chronic toxicity and other health complications such as abdominal pains, nausea, increased cardiac rates, neuro-toxicity, genotoxicity and liver diseases (Ma et al., 2007; Adibah et al., 2019). Tannins exhibits strong biological activities (anti-bacterial, antimicrobial, hepato-protective, antiarrhythmic and immune-modulatory) (Adewale et al., 2021; Singh et al., 2021).

Phenols have strong antioxidant properties and are capable of preventing oxidative damage to biomolecules such as DNA, lipids and proteins (Hassan et al., 2012; Singh et al., 2023). Saponins are immune stimulators (Hassan et al., 2012), antimicrobials (Soetan et al., 2006). Researchers have reported its hypocholesterolemic properties (Cheeke, 2000). Phytates have been reported to be effective in the treatment of wounds and haemorrhage

(Oluwafemi et al., 2021). They are antioxidants capable of reducing the risk of cardiovascular disease, kidney stones, osteoporosis and iron absorption (Shittu; Alagbe, 2021). Excessive ingestion of oxalates can interfere with the absorption of calcium in the body (Varadharajan et al., 2012); Sombié et al. (2011). The results obtained in this research is in consonance with previous findings of Ogbeba et al. (2017); Singh & Bhat (2013). Tobias (2010) reported that the composition of essential oils is influenced by cultivar within the plant variety, climatic conditions and time of harvest. The presence of multiple phyto-constituents in *Prosopis africana* shows that it has notable beneficial effects in maintaining animal's performance, antioxidant and osmotic balance, palatability, retention time of feed, modulating fatty acid in the muscle as well as supporting the intestinal integrity (Singh et al., 2023).

Table 2. Phytochemical constituents of *Prosopis africana* oil.

Constituents	Concentrations (mg/g ⁻¹)
Tannins	201.21
Flavonoids	677.83
Alkaloids	405.90
Saponins	72.62
Phenols	508.28
Phytates	20.10
Oxalates	5.26

Source: Authors, 2023.

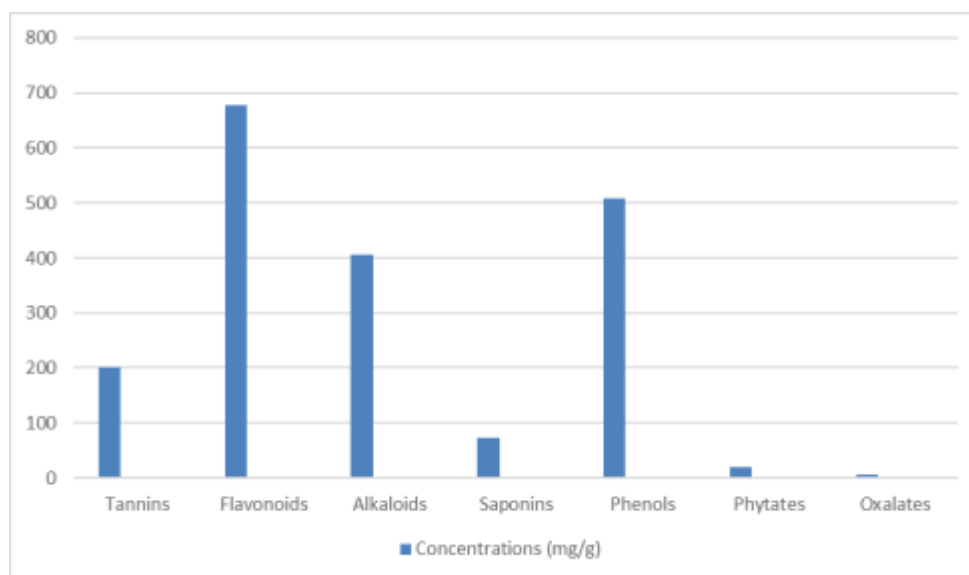
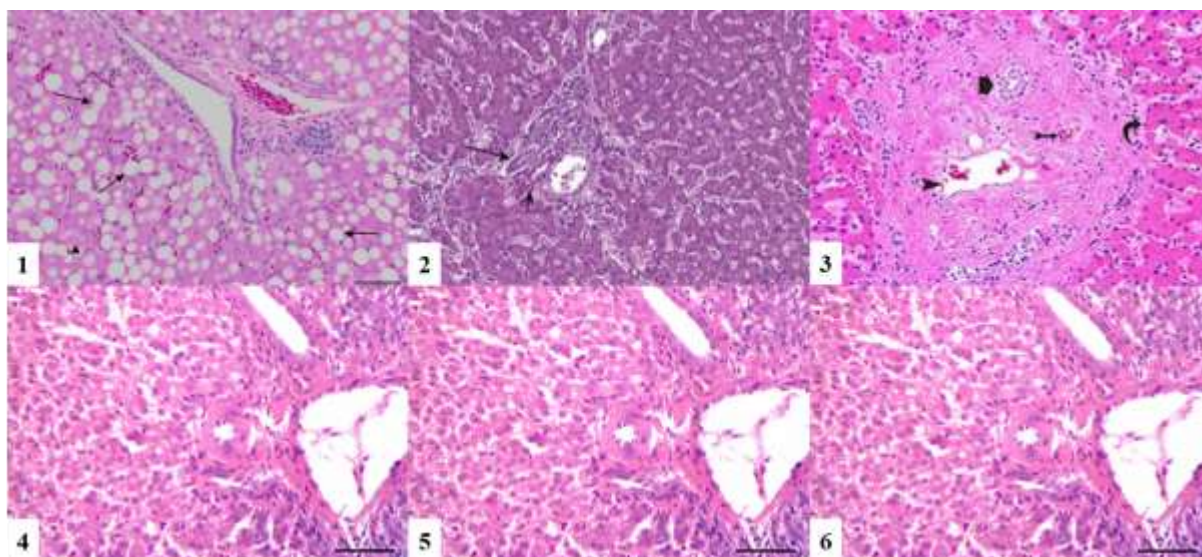


Figure 1. Phyto-constituents in *Prosopis africana* oil. Source: Authors, 2023.

3.2 Histopathology examination of birds fed *Prosopis africana* oil (PRSO)

The examined histopathological liver sections is presented in (Board 1 (Figure 1 at 6) Histopathology). The liver is the largest gland in the body. It is made up of hepatic lobules which are classically described as been hexagonal in structure (Dooley et al., 2002). Birds in Figure 1 (fed 0% PRSO) showed no histopathological signs of any damage; the central vein, blood portal vessels showed no congestion. Figure 2 (fed oxytetracycline) showed mild perivascular lymphocytic aggregation and moderate hepatic sinusoids. Figure 3, 4, 5 and 6 (fed PRSO) revealed normal tissue architecture with normal hepatic lobules, central vein and hepatic sinusoids.



Board 1. Figure 1. Basal diet with no *Prosopis africana* oil (Negative control). Figure 2. Basal diet + Neomycin 1.00 g/kg⁻¹ (Positive control). Figure 3. Basal diet + 200 mg/kg⁻¹ *Prosopis africana* oil (PRSO). Figure 4. Basal diet + 400 mg/kg⁻¹ PRSO. Figure 5. Basal diet + 600 mg/kg⁻¹ PRSO. Figure 6. Basal diet + 800 mg/kg⁻¹ PRSO. Source: Authors, 2023.

According to Harold et al. (2008); Plinio & Broglia (2000), the liver help to detoxify toxic substances, metabolism of micro-nutrients, excretion of bilirubin and synthesis of bile salts. The healthy tissue architecture of birds given *Prosopis africana* oil is evident that both their bioactive components and inclusion levels are safe. PRSO are well known for their beneficial effects on animals, from anti-inflammatory, antioxidant and antimicrobial properties (Agubosi et al., 2022; Oluwafemi et al., 2020). The outcome of this investigation is consistent with the findings of Oloruntola (2019) when phytochemicals (pawpaw leaf and seed meal) were supplemented in the diet of broiler chickens.

Similar findings were made by Samantha et al. (2019) when essential oils were given to broilers that were coccidiosis-challenged. Omokore & Alagbe (2019); Alagbe & Oluwafemi (2019) claimed that bioactive substances found in essential oils may inhibit the pathogenicity features in bacteria, such as flagella, which are important for bacterial adhesion or adherence to any mammalian preference sites. Alagbe (2022) asserts that the liver is crucial for the creation of bile, the metabolism of carbohydrates, and the removal of toxins from an animal's system. The abundance of flavonoids, a significant phytochemical compound could significantly decrease liver tissue degenerative conditions particularly blockages in the liver of animals when compared to the other treatments.

Antibiotics like oxytetracycline are widely used to prevent, control, and treat disease in animals; however, due to their uncontrolled usage and insufficient sensitivity testing before treatment, there are a rising number of incidences of resistance (Adewale et al., 2021). Alagbe et al. (2019) claim that bioactive substances found in essential oils may inhibit the pathogenicity features in bacteria, such as flagella, which are important for bacterial adhesion or adherence to any mammalian preference sites. Alagbe (2022) asserts that the liver is crucial for the creation of bile, the metabolism of carbohydrates, and the removal of toxins from an animal's system.

The abundance of flavonoids, a significant botanical compound could significantly decrease liver tissue degenerative conditions particularly blockages in the liver of animals. Degeneration in liver histopathology has also been observed to be enhanced by contaminants (Soetan; Oyewole, 2009). Infections with bacteria may have caused the severe and mild hepatic damage seen in birds in T2. Despite the fact that antibiotics were administered to the birds in T2, this finding suggests that there may be an antimicrobial resistance issue.

4. Conclusions

It can be concluded that the more antibiotics (oxytetracycline) fed to animals the greater the selective pressure that favours resistant strains. However, *Prosopis africana* essential oil possess several properties – antioxidant, antiviral, anti-inflammatory, anti-depressant, immune-stimulatory, antimicrobial, hepato-protective amongst others, all of which are vital for the performance enhancing effects in livestock's.

5. Acknowledgments

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6. Authors' Contributions

Olujimi John Alagbe: field work and compilation of results, submission, corrections and publication. *O. C. P Agubosi*: field work and compilation of results. *R. A. Oluwafemi*: field work and compilation of results.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Yes applicable.

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