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COMPORTAMIENTO REPRODUCTIVO EN CONEJOS SUPLEMENTADOS CON EXTRACTOS DE TÉ VERDE BAJO UN PROGRAMA DE INSEMINACIÓN ARTIFICIAL

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Presenta:

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“No importa lo oscuro que se ponga el mundo, hay luz en nosotros que nunca se apaga." - *Albus Dumbledore*

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La verdadera fuerza proviene de la voluntad de continuar luchando, incluso cuando todo parece perdido.”-*Loki*

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COMPORTAMIENTO REPRODUCTIVO EN CONEJOS SUPLEMENTADOS CON EXTRACTOS DE TÉ VERDE BAJO UN PROGRAMA DE INSEMINACIÓN ARTIFICIAL[1]

REPRODUCTIVE BEHAVIOR IN RABBITS SUPPLEMENTED WITH GREEN TEA EXTRACTS UNDER AN ARTIFICIAL INSEMINATION PROGRAM[2]

Resumen General

El uso de suplementos naturales como el extracto de Camellia sinensis (té verde) representa una alternativa prometedora para mejorar la eficiencia reproductiva en la cunicultura. Este documento integra dos contribuciones orientadas a evaluar el impacto del extracto del té verde sobre la reproducción cunícola. El primer artículo es una revisión de literatura que sintetiza evidencias sobre los efectos del té verde en la reproducción animal, destacando sus propiedades antioxidantes, su impacto sobre la calidad espermática, los ciclos hormonales, la ovulación y la embriogénesis, así como sus interacciones con el microbioma intestinal y su papel potencial como suplemento funcional. La revisión también discute sus mecanismos de acción y beneficios en diferentes especies, incluyendo modelos en conejos, y señala que los efectos dependen de las distintas dosis. El segundo artículo es un estudio experimental original que evaluó el efecto de la suplementación con extracto de té verde (75 mg/kg peso vivo) en conejos bajo un programa de inseminación artificial. Se utilizaron 80 conejas y 8 machos fértiles, asignados a cuatro tratamientos con distintas combinaciones de suplementación en machos y hembras. En hembras, el té verde mejoró significativamente la receptividad sexual, la prolificidad y el peso de camada al destete. En machos, la suplementación mejoró la concentración espermática, la motilidad progresiva, los parámetros cinéticos del movimiento espermático y el número de dosis potenciales para inseminación.

Palabras clave: antioxidantes, calidad seminal, estrés oxidativo, fertilidad, inseminación artificial, reproducción cunícola, té verde.

General Abstract:

Natural supplements such as Camellia sinensis (green tea) extract represent a promising alternative for improving reproductive efficiency in rabbit breeding. This article integrates two contributions to evaluate green tea extract's impact on rabbit reproduction. The first article is a literature review that synthesizes evidence on the effects of green tea on animal reproduction, highlighting its antioxidant properties, its impact on sperm quality, hormonal cycles, ovulation, and embryogenesis, as well as its interactions with the gut microbiome and its potential role as a functional supplement. The review also discusses its mechanisms of action and benefits in different species, including rabbit models, and notes that the effects depend on different doses. The second article is an original experimental study that evaluated the effect of green tea extract supplementation (75 mg/kg body weight) in rabbits undergoing an artificial insemination program. Eighty males and eight fertile males were used and assigned to four treatments with different supplementation combinations in males and females. In females, green tea significantly improved sexual receptivity, prolificacy, and litter weight at weaning. In males, supplementation improved sperm concentration, progressive motility, kinetic parameters of sperm movement, and the number of potential doses for insemination.

Keywords: antioxidants, artificial insemination, fertility, green tea, oxidative stress, rabbit reproduction, semen quality.

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1. INTRODUCCIÓN GENERAL

La reproducción eficiente de conejos es crucial para satisfacer su demanda y mejorar la rentabilidad en los sistemas de producción. En este contexto, la inseminación artificial (IA) es una herramienta para mejorar los resultados reproductivos, potenciando la mejora genética y el control de enfermedades (Quintela et al., 2023). No obstante, la eficiencia de la IA depende de factores como la salud general y el estado nutricional de los animales reproductores.

En conejos, el estrés oxidativo es un factor crítico que afecta el rendimiento reproductivo, particularmente bajo protocolos de IA donde prevalecen factores estresantes, debido a la manipulación física durante el proceso, los cambios en su rutina diaria, y el ambiente donde se realiza el procedimiento (Soliman & El-Sabrout, 2019).

La investigación ha demostrado que el estrés oxidativo, condición caracterizada por un desequilibrio entre las especies reactivas de oxígeno (ROS) y las defensas antioxidantes puede ser inducido por diversos factores ambientales y fisiológicos (Giraud-Billoud et al., 2023). Por ejemplo, el estrés calórico disminuye la eficiencia reproductiva en conejos al aumentar el estrés oxidativo, lo que afecta negativamente la calidad seminal y el estado antioxidante (Abubakar & Olubisi, 2018). En estudios con conejos Nueva Zelanda Blanco se ha demostrado que el estrés calórico conduce a una mayor peroxidación lipídica y disminución de la capacidad antioxidante, aunque la suplementación con antioxidantes como el zinc, L-carnitina, vitamina C, nanopartículas de selenio y espirulina pueden mitigar estos efectos (El-Gindy et al., 2023; El-Ratel et al., 2023; El-Tohamy et al., 2012; Imbabi et al., 2023; Rehman et al., 2024).

Adicionalmente, el estrés oxidativo se ha relacionado con una disminución de la función ovárica y la fertilidad, debido a la reducción de la calidad del folículo y la producción de hormonas (Shi et al., 2016). Se ha sugerido que el estrés oxidativo dirige la declinación relativa a la edad de la vida fértil, y desempeña un papel importante durante la gestación, el parto normal y en el inicio de un parto prematuro (Ruder et al., 2008).

En años recientes se ha explorado la suplementación con compuestos naturales y funcionales como una estrategia para mitigar los daños causados por el estrés oxidativo y que puedan optimizar la salud y eficiencia reproductiva de los animales. El té verde (*Camellia sinensis*), conocido por sus propiedades antioxidantes, ha sido estudiado por su potencial para reducir el estrés oxidativo. Sus componentes bioactivos, especialmente los polifenoles como las catequinas, podrían desempeñar un papel crucial en la mejora del comportamiento reproductivo y la respuesta a los programas de IA (Li et al., 2021).

El té verde tiene efectos benéficos en la salud y el bienestar de varias especies. En bovinos, la suplementación con extracto de té verde en diluyentes de semen ha mejorado la viabilidad espermática, la motilidad y la tasa de gestación, lo que sugiere su potencial en la mejora de la eficiencia reproductiva (Susilowati et al., 2021). De manera similar, en conejos, la suplementación con extractos naturales, incluyendo polifenoles, mejora el estado antioxidante e influye positivamente en su reproducción (Vizzarri et al., 2020). No obstante que los efectos del té verde en la reproducción de conejos son complejos, algunos estudios indican beneficios sobre la calidad del esperma y la embriogénesis (Svoradová et al., 2021), otros mencionan que su impacto depende de la dosis y que podría afectar negativamente la calidad espermática a ciertas concentraciones (Baláži et al., 2022).

A pesar del interés en la suplementación con extractos naturales y que los estudios proponen que el té verde influye positivamente en diversos aspectos de la salud, su impacto específico en la reproducción de conejos no ha sido completamente investigado. Por lo tanto, el objetivo de esta investigación fue evaluar los efectos de la suplementación con extractos de té verde alto en polifenoles sobre el comportamiento sexual, fertilidad y prolificidad de conejas bajo un programa de IA.

En el Capítulo 2 se presenta un artículo de revisión donde se explica el efecto del té verde en la reproducción cunícola. En el Capítulo 3 se presenta un artículo de investigación en donde se muestran los efectos del té verde sobre parámetros reproductivos en hembras y machos, así como sus interacciones.

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2. EFECTO DEL TÉ VERDE EN LA REPRODUCCIÓN CUNÍCOLA: UNA REVISIÓN

EFFECT OF GREEN TEA ON RABBIT REPRODUCTION: A REVIEW

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SUMMARY

Background. Reproduction in rabbit enterprises is crucial for productivity, but stress and nutritional deficiencies can affect it. Green tea, known for its antioxidant properties, has been the subject of studies in various animal species, suggesting possible benefits in reproductive health. **Objective**. To review the effects of green tea on rabbit reproduction, exploring its possible mechanisms of action. **Main findings**. It was found that bioactive compounds in green tea could improve semen quality, regulate hormonal cycles, and protect against oxidative stress, improving rabbits' fertility. However, the results vary depending on the dosage. **Conclusions**. Although green tea has potential as a supplement to improve rabbit reproduction, further research is required to determine optimal doses, evaluate its long-term impact, and consider possible side effects or interactions with other nutrients.

Keywords: Green tea; rabbit reproduction; oxidative stress; antioxidants; semen quality; fertility.

RESUMEN

Antecedentes. La reproducción en empresas cunícolas es crucial para la productividad, pero factores como el estrés y las deficiencias nutricionales pueden afectarla. El té verde, conocido por sus propiedades antioxidantes, ha sido objeto de estudios en diversas especies animales, sugiriendo posibles beneficios en la salud reproductiva. **Objetivo**. Revisar los efectos del té verde en la reproducción cunícola, explorando sus posibles mecanismos de acción. **Hallazgos principales**. Se encontró que los compuestos bioactivos del té verde, pueden mejorar la calidad del semen, regular los ciclos hormonales y proteger contra el estrés oxidativo, lo que podría mejorar la fertilidad en conejos. Sin embargo, la los resultados varían según la dosificación. **Conclusiones**. Aunque el té verde presenta potencial como suplemento para mejorar la reproducción cunícola, se requieren más investigaciones para determinar las dosis óptimas y evaluar su impacto a largo plazo, además de considerar posibles efectos secundarios o interacciones con otros nutrientes.

Palabras clave: Té verde; reproducción cunícola; estrés oxidativo; antioxidantes; calidad seminal; fertilidad.

INTRODUCCIÓN

La cunicultura juega un papel importante en la industria pecuaria, particularmente para uso cárnico, pelaje y para fines de laboratorio, por lo que mejorar la eficiencia reproductiva es crucial para aumentar la productividad y la viabilidad económica. Lo anterior implica optimizar las técnicas de reproducción, como la inseminación artificial (IA), y mejorar la salud reproductiva tanto en machos como en hembras (Piza et al., 2021).

El té verde, derivado de las hojas de *Camellia sinensis*, es valorado por sus beneficios para la salud, atribuidos a su alto contenido de catequinas, un tipo de polifenol con propiedades antioxidantes. Esta revisión analiza el impacto del té verde en la reproducción cunícola, destacando su potencial para mejorar la salud reproductiva y el rendimiento. Las catequinas del té verde, especialmente la epigalocatequina-3-galato (EGCG), son cruciales para mitigar el estrés oxidativo, un factor significativo que afecta la fertilidad en machos y hembras. El estrés oxidativo, causado por un desequilibrio entre especies reactivas de oxígeno (ROS) y antioxidantes, puede dañar el ADN y comprometer la motilidad de los espermatozoides y la maduración de los ovocitos, afectando los resultados reproductivos (Rahman et al., 2018; Roychoudhury et al., 2017).

Este capítulo se enviará a la revista científica *Tropical and Subtropical Agroecosystems.*

Estudios en modelos animales, incluidos conejos y ratones, indican que el té verde puede influir positivamente en la calidad del esperma, los perfiles hormonales y la embriogénesis. En conejos, se ha observado una mejora en la motilidad espermática y en la embriogénesis, sugiriendo su potencial como suplemento natural (Svoradová et al., 2021). El té verde ha contrarrestado la toxicidad reproductiva inducida por toxinas ambientales, mejorando la calidad del esperma en ratones (Silviani et al., 2022). También se ha señalado su papel en el envejecimiento reproductivo, extendiendo la vida reproductiva (Hoque et al., 2023). Las propiedades quimiopreventivas del té verde en el contexto de cánceres reproductivos subrayan sus posibles aplicaciones terapéuticas. Sin embargo, la traducción de estos beneficios a entornos clínicos es aún limitada, por lo que se requieren más investigaciones para establecer pautas sobre su uso en salud reproductiva (Kamal et al., 2021). Los efectos multifacéticos del té verde sobre la salud reproductiva, que incluyen acciones antioxidantes y antiinflamatorias, resaltan su relevancia para la investigación en la reproducción cunícola (Idowu, 2017).

Composición del té verde

Los componentes principales del té verde incluyen polifenoles, alcaloides, aminoácidos, vitaminas y minerales; entre estos, los polifenoles, particularmente las catequinas, son los más destacados, con cuatro tipos principales identificados: epicatequina (EC), epigalocatequina (EGC), galato de epicatequina (ECG), y galato de epigalocatequina (EGCG), siendo este último el componente más activo (Joseph et al., 2024; Sarma et al., 2023). Estas catequinas son responsables de las propiedades antioxidantes, anticancerígenas y antimicrobianas del té verde, contribuyendo a sus efectos promotores de la salud (Ratnani & Malik, 2022).

En salud animal, las catequinas han demostrado beneficios potenciales como reducir la obesidad y el riesgo de diabetes tipo II, mejorar la salud cardiovascular y ofrecer protección contra la radiación ultravioleta (Mansoori et al., 2022; Soboleva et al., 2023). Se ha encontrado que las catequinas inducen apoptosis en células cancerosas, modulan alteraciones epigenéticas y promueven la diferenciación de las células leucémicas, destacando su potencial terapéutico en neoplasias hematológicas (Della et al., 2023). A pesar de estos beneficios, la biodisponibilidad de las catequinas es limitada, debido a su mala absorción en el tracto digestivo, aunque el metabolismo mediado por la microbiota intestinal puede potenciar su absorción (Andrade et al., 2024).

El té verde también contiene alcaloides como la cafeína, teobromina y teofilina, que imparten un efecto estimulante y contribuyen a su sabor y amargor característicos (Ratnani & Malik, 2022). El aminoácido teanina es otro componente conocido por sus efectos calmantes y su capacidad para mejorar el enfoque mental (Karunaratne et al., 2022). Adicionalmente, el té verde es rico en vitaminas A, B1, B2, B3, C y E, que aumentan su potencial antioxidante y contribuyen a diversos beneficios para la salud, incluyendo el apoyo inmunológico y la salud de la piel (Khatoon, 2023). Los minerales y oligoelementos presentes en el té verde como calcio, potasio, magnesio, sodio, hierro, manganeso y zinc, aumentan aún más su valor nutricional, apoyando la salud y el bienestar general (Długaszek & Mierczy, 2023). La presencia de polisacáridos y componentes volátiles también se suma a las actividades farmacológicas del té verde, incluyendo efectos hipoglucemiantes y neuroprotectores (Zhao et al., 2022).

A pesar de sus numerosos beneficios, la composición del té verde también puede conducir a efectos adversos si se consume en exceso, como hepatotoxicidad y alteraciones gastrointestinales (Sarma et al., 2023). La composición diversa del té verde lo convierte en un alimento funcional con un importante potencial nutracéutico y farmacéutico, como lo demuestra su creciente mercado e inclusión en diversos productos de salud (Khatoon, 2023). En general, la composición integral del té verde, con su variedad de compuestos bioactivos, lo convierte en un tema de extensa investigación e interés a nivel mundial.

Mecanismos de acción

Los mecanismos de acción del té verde son multifacéticos e involucran varias vías biológicas. Los polifenoles que lo componen pueden interactuar con los productores de ROS como Lipoxigenasa, amortiguando así la producción de estas especies y el estrés oxidativo (Rajak et al., 2023). Las catequinas del té verde modulan el sistema antioxidante endógeno, mejoran la reparación del daño oxidativo del ADN y promueven la apoptosis en células con daño genético, lo que es particularmente beneficioso en poblaciones expuestas al daño genotóxico inducido por metales (García-Rodríguez et al., 2023). El té verde también ha demostrado efectos antiinflamatorios significativos al inhibir la producción excesiva de citocinas, lo cual es crucial en el manejo de enfermedades neurodegenerativas y déficits cognitivos (Gonçalves et al., 2023). Las catequinas también exhiben propiedades antibacterianas a través de mecanismos como la alteración de la pared celular, la inhibición de enzimas intracelulares y la quelación del hierro, con compuestos galoilados como EGCG mostrando la mayor actividad antibacteriana (Zhang et al., 2023). En términos de salud cardiovascular, los flavonoides del té verde potencian la producción de óxido nítrico, reducen los niveles de colesterol e inhiben la agregación plaquetaria, reduciendo así el riesgo de enfermedades cardiovasculares (Godos et al., 2024).

Los efectos antihipertensivos del té verde se atribuyen a su capacidad para modular el sistema renina-angiotensina-aldosterona, mejorar la síntesis de óxido nítrico y suprimir la inflamación, que colectivamente ayudan a disminuir la presión arterial y mejorar la rigidez arterial (Szulińska et al., 2017). El té verde también exhibe efectos antidiabéticos al mejorar la tolerancia a la glucosa y la resistencia a la insulina, en parte a través de la regulación de la expresión de dipeptidil peptidasa-4 (DPP4) en el tejido adiposo, que está mediada por la inhibición del factor nuclear kappa B (NF-κB) (Choi et al., 2023). Las catequinas del té verde pueden oxidar sulfuro de hidrógeno a polisulfuros, que son potentes antioxidantes y provocan efectos citoprotectores, lo que sugiere un papel pro-oxidante que contribuye a sus beneficios para la salud (James et al., 2023).

Mecanismos de acción en la reproducción

El mecanismo primario por el cual el té verde beneficia a la reproducción es a través de sus potentes propiedades antioxidantes, que ayudan a mitigar el estrés oxidativo, factor importante en los trastornos reproductivos. El estrés oxidativo, puede dañar la integridad de los espermatozoides y ovocitos, lo que lleva a la infertilidad. Las catequinas del té verde, especialmente EGCG, reducen la producción de ROS, mejorando así la concentración espermática, la motilidad, la morfología y la integridad del ADN, además de mejorar la maduración y calidad de los ovocitos (Rahman et al., 2018).

Las actividades estrogénicas y antiestrogénicas del té verde contribuyen a sus beneficios reproductivos, ofreciendo posibles efectos protectores contra la pérdida ósea y problemas cardiovasculares, a la vez que se explora por su papel en la quimioprevención del cáncer (Kayami, 2019). Su consumo aumenta la concentración y viabilidad de los espermatozoides sin efectos adversos sobre la salud reproductiva, hepática o renal, aunque algunos cambios estructurales sutiles en los órganos reproductivos justifican una mayor investigación (Joseph et al., 2024).

Los polifenoles del té verde ejercen efectos significativos sobre el microbioma intestinal, lo que a su vez puede influir en la salud reproductiva. La interacción entre los polifenoles y la microbiota intestinal es bidireccional; pueden modular la composición de las bacterias intestinales, promoviendo especies beneficiosas como *Bifidobacterium*, a la vez que inhiben otras como las del phylum *Actinobacteria*, *Bacteroides*, y *Firmicutes*, a excepción de *Lactobacillus* (Bond & Derbyshire, 2019; Choi et al., 2023). Esta modulación puede corregir la disbiosis microbiana asociada a afecciones como la obesidad y la inflamación, que están vinculadas a problemas de salud reproductiva (Bond & Derbyshire, 2019; Pérez-Burillo et al., 2021). El metabolismo de los polifenoles por las bacterias intestinales da como resultado metabolitos bioactivos que potencian las actividades antioxidantes, reduciendo potencialmente el estrés oxidativo (Choi et al., 2023; Rahman et al., 2018). Estas propiedades antioxidantes del té verde, facilitadas por su interacción con el microbioma intestinal, tienen un papel terapéutico prometedor en la mejora de la salud reproductiva al mitigar el estrés oxidativo y apoyar la calidad de los gametos (Li et al., 2019; Rahman et al., 2018). Por lo tanto, la interacción entre el té verde, la microbiota intestinal y la salud reproductiva subraya el potencial de las intervenciones dietéticas en el manejo de los problemas reproductivos.

Efectos del té verde en la salud reproductiva animal

Las catequinas del té verde, han sido ampliamente estudiadas por sus efectos potenciales sobre la salud reproductiva animal. Se ha demostrado que estos compuestos, mitigan el estrés oxidativo, un factor significativo que afecta la fertilidad al dañar la calidad de los espermatozoides y ovocitos a través de ROS (Rahman et al., 2018; Roychoudhury et al., 2017).

En machos, se ha informado que mejoran la calidad del semen al mejorar la concentración espermática, la motilidad, la morfología y la reducción del daño al ADN, aumentando así potencialmente las tasas de fecundación (Parvizi et al., 2024; Rahman et al., 2018). Por ejemplo, se ha encontrado que EGCG mejora la calidad del esperma de toro durante la criopreservación al aumentar la capacidad antioxidante y reducir la peroxidación lipídica y el daño del ADN (Parvizi et al., 2024). De manera similar, en los búfalos, la suplementación dietética con extracto de té verde mejoró la competencia de desarrollo de ovocitos y aumentó el número de complejos acumulus-ovocito de alta calidad, probablemente debido al estado antioxidante potenciado (Benitez et al., 2024). No obstante estos resultados favorables del té verde no son universales; en algunos estudios, como los que involucran machos de ratas albinas, se encontró que el extracto de hoja de té verde reduce los niveles de testosterona y altera los niveles de gonadotropina, lo que lleva a una inhibición de la espermatogénesis y una disminución de la motilidad espermática (Das & Karmakar, 2015).

El uso del té verde también ha mostrado potencial en la modulación de las hormonas reproductivas, como se observa en un estudio en ratas con síndrome de ovario poliquístico, donde el extracto de té verde influyó en los niveles de gonadotropinas y hormonas sexuales (Sadoughi & Rahbarian, 2017). En otro estudio con ratones hembra, el consumo de té verde extendió el período reproductivo activo y mejoró los resultados de fertilidad, como el aumento del tamaño de la camada y mayores pesos de nacimiento y destete de la descendencia, asimismo mejoró la salud ovárica al mantener los folículos ováricos sanos y aumentar los niveles séricos de inmunoglobulina, lo que indica una mejor inmunidad (Hoque et al., 2023). Por otro lado, en codornices, la suplementación dietética con té verde en polvo mejoró los rasgos reproductivos, incluyendo el peso del huevo y las características internas (Lanja & Edrees, 2022).

Efectos del té verde en la reproducción cunícola

En hembras, el extracto de té verde mejora la actividad ovárica, la calidad de los ovocitos y la producción de embriones *in vitro*, específicamente, la administración oral de polifenoles del té verde a 200 mg kg^{-1} de peso corporal mejoró el número de ovocitos y las tasas de gestación (El-Ratel et al., 2017). Las propiedades antioxidantes del té verde, que son más eficaces que las de las vitaminas C y E, reducen el estrés oxidativo, un problema común que afecta la calidad de los gametos (Rahman et al., 2018). También se ha observado que el té verde extiende la vida reproductiva en ratones viejos, mostrando beneficios potenciales similares en conejos, al mantener folículos ováricos sanos y mejorar la inmunidad (Hoque et al., 2023).

Un estudio mostró que la suplementación con té verde en las dietas de machos condujo a una reducción en la ganancia de peso vivo y un deterioro en la calidad del esperma, particularmente a dosis más bajas de 5 g por 100 kg de alimento, lo que disminuyó la concentración y motilidad de los espermatozoides, aunque una dosis mayor de 20 g fue neutra en sus efectos (Baláži et al., 2022). Por el contrario, otro estudió demostró que un mayor nivel de suplementación de 6 g kg^{1} de té verde, fue efectiva para potenciar estos parámetros, indicando su potencial como tratamiento de apoyo en el manejo nutricional para mejorar la calidad del semen (Sheteifa & Morsy, 2014). Las propiedades antioxidantes de las catequinas del té verde son fundamentales para reducir las ROS, que de otro modo pueden conducir a estrés oxidativo y afectar la integridad del esperma (Rahman et al., 2018).

Si bien los efectos del té verde sobre el rendimiento reproductivo en conejos son prometedores, se necesita más investigación para explorar sus mecanismos y optimizar su uso en combinación con otros extractos naturales para mejorar los resultados reproductivos y optimizar su uso en los sistemas de producción cunícola.

Té verde y otros suplementos nutricionales en la reproducción cunícola

Otros suplementos nutricionales, como una combinación de antioxidantes como la vitamina E, el selenio y la L-carnitina, mejoran la fertilidad masculina al mejorar la calidad y motilidad de los espermatozoides, así como aumentar las tasas de nacimientos vivos y de gestación. Estos suplementos funcionan mitigando el estrés oxidativo, que es un factor común que perjudica la salud reproductiva (Mora-Esteves & Shin, 2013). El polen de palmera datilera ha mostrado efectos positivos sobre el rendimiento reproductivo en conejos, mejorando significativamente las tasas de gestación, las características de la camada y los perfiles hormonales, al tiempo que mejora la capacidad antioxidante y los marcadores de respuesta inmunológica (Baagar, et al., 2022). Se investigó la quercetina, un flavonoide, por su potencial para mejorar el estado antioxidante de los conejos, pero no alteró significativamente el perfil antioxidante ni la salud clínica de los animales, resultando en una eficacia limitada en cuanto a la mejora de los resultados reproductivos bajo las condiciones del estudio (Vicente-Carrillo et al., 2024).

El uso general de extractos naturales, incluyendo polifenoles, se ha asociado con mejoras en la calidad del esperma y marcadores antioxidantes en conejos machos (Vizzarri et al., 2018). Adicionalmente, otros antioxidantes naturales como el jengibre mejoran la calidad del semen y el estrés oxidativo, lo que indica beneficios potenciales para la salud reproductiva (El-Ratel et al., 2021). Si bien tanto el té verde como otros antioxidantes mejoran la salud reproductiva a través de sus propiedades antioxidantes, el té verde específicamente ha mostrado eficacia en estudios con animales, para mejorar los resultados reproductivos en diferentes especies (Benitez et al., 2024; Sheteifa & Morsy, 2013).

Beneficios adicionales del té verde en la salud cunícola

El té verde aporta diversos beneficios adicionales a la salud general de los conejos, promoviendo su bienestar y calidad de vida. Este ingrediente ha mostrado una mejora en el estado antioxidante y la respuesta inmune de conejos al destete, que son críticos para reducir el estrés oxidativo y aliviar el estrés del destete, protegiéndolos en ese periodo vulnerable (Yin et al., 2024). Por otro lado, también proporciona efectos neuroprotectores, antiinflamatorios y antimicrobianos, que pueden contribuir al control de enfermedades y mejorar los resultados de salud en los conejos (Natarajan et al., 2019).

Los polifenoles del té verde se han destacado por su capacidad para mejorar la inmunidad, lo cual es crucial para prevenir enfermedades y promover la longevidad (Bhushan et al., 2017). Los beneficios inmunológicos del té verde, particularmente su impacto en las células, apoyan aún más su papel en reforzar el sistema inmunológico, convirtiéndolo en un valioso suplemento dietético para mantener la salud del conejo (Huynh, 2017). Su capacidad para enriquecer la microbiota intestinal beneficiosa y mejorar la morfología intestinal sugiere posibles beneficios indirectos sobre el crecimiento y la productividad al mejorar la absorción de nutrientes y la salud intestinal (Yin et al., 2024).

Algunos estudios han demostrado que el consumo de té verde puede conducir a la reducción de peso y mejorar los perfiles lipídicos séricos en conejos. Por ejemplo, con una dieta alta en colesterol complementada con té verde se mostró una reducción en el peso corporal e inhibieron aumentos en los niveles de colesterol total, lipoproteínas de baja densidad y lipoproteínas de muy baja densidad en comparación con los grupos control (Huang et al., 2022; Kongchian et al., 2020).También se redujeron los niveles de colesterol sérico en conejos alimentados con colesterol, aunque no afectó los niveles de lipoproteínas de alta densidad (Aftabuddin et al., 2016). Por otro lado, el té verde ha demostrado efectos hepatoprotectores, ya que mitigó los efectos hepáticos adversos de la ciclosporina en conejos, preservando la estructura y función normal de las células hepáticas (Fetouh & Ibrahim, 2013).

Consideraciones de la administración de té verde

Al considerar la administración del té verde en la dieta de conejos, se deben tomar en cuenta varios factores, entre ellos posibles efectos secundarios y formas de administración. Al ser administrado principalmente en forma de polvo, influye en diversos parámetros fisiológicos en conejos. Por ejemplo, puede reducir el aumento de peso y afectar la calidad del esperma, con dosis más bajas disminuyendo la concentración y motilidad espermática, mientras que dosis más altas parecen neutrales al respecto (Baláži et al., 2022). No obstante, la administración a largo plazo de té verde, se ha relacionado con un transporte inverso de colesterol alterado y un aumento de la rigidez vascular, lo que podría elevar el riesgo de lesiones ateroscleróticas (Hunjadi et al., 2021).

A pesar de los beneficios mostrados, existen preocupaciones sobre la hepatotoxicidad de las catequinas, aunque estudios sobre té verde fermentado no han demostrado efectos tóxicos significativos a dosis de hasta 2.0 g kg^{-1} en roedores (Lee et al., 2019).

CONCLUSIÓN

El té verde, reconocido por su rico contenido en catequinas y propiedades antioxidantes, ha demostrado un impacto positivo en la reproducción cunícola. No solo mejora la salud reproductiva al mitigar el estrés oxidativo, sino que también influye favorablemente en parámetros clave como la calidad del esperma y la ovulación en conejas. Sin embargo, su administración en dietas de conejo debe manejarse cuidadosamente para evitar posibles efectos adversos, particularmente sobre la salud reproductiva y la función cardiovascular

Es fundamental llevar a cabo investigaciones adicionales para determinar las dosis óptimas y evaluar combinaciones, con otros extractos de plantas. Esto permitirá maximizar los beneficios del té verde en la reproducción cunícola y mitigar los posibles riesgos asociados.

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animals

Article

3. Reproductive behavior in rabbits supplemented with green tea extracts under an artificial insemination program

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Abstract: This study evaluated the effects of *Camellia sinensis* (green tea) extract supplementation on reproductive performance in does and semen quality in bucks under an artificial insemination program. Eighty does and eight bucks were assigned to four treatments: T1) both sexes supplemented; T2) only females; T3) only males; T4) no supplementation. The extract (75 mg/kg BW) was administered via drinking water five days weekly. In females, supplementation improved sexual receptivity (+38.5%; $p = 0.003$), total and live litter size (both ~+19%), and litter weight at weaning (+12.3%). In males, sperm concentration per ejaculate, velocity (VSL), linearity (LIN), and AI dose yield increased significantly ($p < 0.05$). These results suggest that green tea polyphenols can enhance reproductive efficiency in rabbits without compromising health.

Keywords: antioxidants, rabbit, Camellia sinensis, artificial insemination, reproductive performance, semen quality.

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1. Introduction

The study of reproductive behavior in rabbits, particularly under the influence of supplements such as green tea extracts, is a growing area with important implications for reproductive production and biotechnology. Green tea (*Camellia sinensis*), renowned for its antioxidant properties, has been shown to improve ovarian activity and oocyte quality and increase fertilization rates in female rabbits, suggesting its potential to optimize efficiency in artificial insemination (AI) programs [1].

However, the effects of green tea are not always positive; in male rabbits, its supplementation has been associated with reduced weight and impaired sperm quality, indicating a complex interaction that may be dose-dependent [2].

Supplementing rabbits with natural extracts, including polyphenols, improves antioxidant status. However, its direct impact on reproductive performance remains uncertain, requiring further research [3,4]. Incorporating green tea extracts into AI programs could help mitigate the adverse effects of environmental factors such as heat [5]. Despite promising findings, variability in responses between different studies highlights the need to investigate optimal doses and combinations to improve reproductive parameters without affecting animal health [6]. Therefore, the objective of the present study was to evaluate the effect of green tea under an AI program.

This chapter will be submitted to the scientific journal *Animals*.

2. Materials and Methods

2.1. Experimental Location

The study was developed in the "Conejos" Scientific Research Center of the State of Mexico A.C. (COCICEMAC), located in San Miguel Coatlinchán, Texcoco de Mora, Mexico, at 19º 27′ N and 98º 53′ W, at 2240 meters above sea level, with a temperature of 15.9 °C and an average annual rainfall of 645 mm [7].

2.2. *Animals, treatments, and experimental design*

Eighty New Zealand White females and eight mature fertile male rabbits of the same breed were used. The reproductive program was AI. The female rabbits were randomly assigned to one of four experimental treatments as follows: 1) supplementation with green tea extracts administered in the drinking water at a rate of 75 mg kg^{-1} of live weight, using males also supplemented with the same dose; 2) supplementation in female rabbits, but not in males; 3) no supplementation in females, but in males; 4) no supplementation in females and males (control group). The experimental design was completely randomized with 20 repetitions per treatment, with the rabbit as the experimental unit. In females and males, supplementation was performed five consecutive days each week. Figure 1 shows the experimental design used in this study.

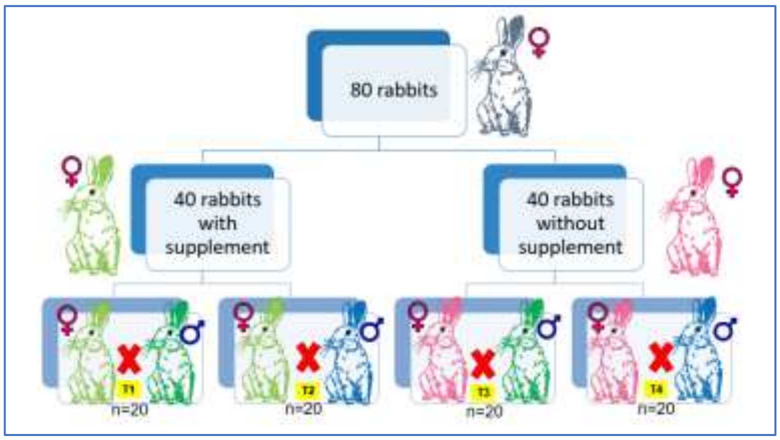

Figure 1.Schematic of the experimental design and distribution of treatments in rabbits.

2.3. *Handling and administration of green tea extracts*

A standardized dry extract of *Camellia sinensis* plant leaves was used, with a high polyphenol content (90%), provided to both females and males in the drinking water at a dose of 75 mg kg^{-1} of body weight. Rabbits on the green tea supplementation regimen underwent a one-month adaptation period before starting the experimental phase. Subsequently, from the beginning to the end of the experiment, the extracts were administered for five consecutive days, from Monday to Friday, followed by two days of rest.

To calculate the green tea extract dose of 75 mg kg^{-1} of body weight per day, females and males were weighed using a digital scale on Saturdays of each week. Based on this weight, the amount of extract to be provided each day during the week was calculated. The weighing of the green tea extract provided was carried out using an electronic scale. The administration of the extract on the scheduled days was performed between 7:00 and 8:00 a.m.

The respective doses for each male and female were diluted in 150 ml of drinking water and administered to semi-automatic drinkers. Automatic drinkers provided free access to water for all breeders at all times.

2.4. Facilities and environment

The animals were housed in a 10 m wide and 25 m long natural environment double-pitched shed, with thermal insulation on the roof, walls, and open side windows. The female rabbits were kept individually in European-style galvanized metal cages measuring 75 cm long x 60 cm wide x 40 cm high. In comparison, the males were housed in cages measuring 45 cm x 60 cm x 40 cm, arranged in a flat-deck system, and provided with automatic nipple-type drinkers and English-style hopper feeders. The nests used were made of galvanized sheet metal measuring 55 cm x 30 cm x 25 cm, with a plywood floor. Both females and males received natural light throughout the experiment.

2.5. Feeding management

A commercial pellet feed (Tepexpan®; Breeding Rabbit) was used with 17.4% crude protein, 5.3% fat, 15.0% crude fiber, 8.0% ash, 44.2% nitrogen-free extract, 1.2% calcium, 0.7% phosphorus, 2608 kcal of digestible energy kg^{-1}, and 10.1% moisture. Breeding does, from mating until the day of palpation, receive restricted feed at 150 g day^{-1}, while pregnant and lactating receive feed ad libitum. Male rabbits were limited to 150 g day^{-1} of feed.

2.6. Reproductive management in males and females

Rabbits were managed under an AI program and a semi-intensive reproduction rhythm until they had completed 1 to 2 births per rabbit. Inseminations were performed on Mondays and Fridays of each week. Biostimulation was performed to synchronize estrus by changing cages eight hours before AI.

Male rabbits were managed under an extensive reproduction rhythm, allowing them only one work session per week with two ejaculations in each session. Primiparous and lactating multiparous rabbits from all treatments were inseminated between days 10 and 12 after birth. Between 10 and 12 days after insemination, rabbits were palpated abdominally for pregnancy diagnosis, and those who were not pregnant were inseminated again. Those diagnosed as positive were placed in nests at 28 days of gestation and provided with oat straw for nest construction. Weaning took place on day 30 after delivery. The breeders were kept according to the treatments in continuous lines of cages or in bands to facilitate handling.

2.7. Semen collection protocol, semen quality assessments, and artificial insemination.

Sperm extractions were performed once a week on each male. An artificial vagina containing 45 °C water was used for semen extraction with the help of a support rabbit. Once the ejaculate was obtained, it was placed in a 32 °C water bath for evaluation. When the samples were presented in gel form, this was removed from the collection tube. Seminal volume was measured directly in graduated collection tubes at a rate of 0.2 mL. Semen pH was measured using pH indicator paper test strips, with values ranging from 1 to 12 in 1-degree intervals (Dual Tint®).

A computerized semen analysis system (CASA, appendices 1 and 2) was used for sperm quality determinations. The system was configured according to the recommendations for rabbit semen evaluation. Each semen sample was diluted 1:5, and a 50 μL aliquot was taken and mixed with 250 μL of phosphate-buffered saline (Dulbecco A; Oxoid Inc., UK). A second dilution was then made with Viadent stain (Hoechst 33258) 1:1, for which 100 μL of the previous dilution was added to 100 μL of Viadent. Viadent stain contains bisbenzimide trihydrochloride and uses fluorescent light to determine the number of nonviable cells since only cells with damaged membranes are stained. An aliquot (±3 μL) of the final dilution was taken and subjected to the CASA computerized semen analysis system (IVOS II, v 1.7, Hamilton Thorne Research, Beverly, MA). For analysis, samples were incubated for 2 min at 37°C inside the CASA in a 20-micron deep Leja (disposable fixed-depth slide). Multiple fields (up to 60 sperm per field) were analyzed, and at least 400 sperm were recorded for motility characteristics in each sample.

For AI, the rabbits were individually restrained in the supine position and injected with 0.8 mL of diluted fresh homospermic semen. Immediately after insemination, ovulation was induced by intramuscular administration of 20 µg of gonadorelin (GnRH; Sanfer®).

2.8. Sanitary management

A sanitary program was carried out in the maternity and male units, which included flaming, washing, and disinfection prior to the start of the research. A blowtorch, a pressure washer, and quaternary ammonium salts (Bactosan Spray; Química pH) were used for disinfection. The excrements of the units were removed every week. In addition, at the beginning of the experiment and before starting the adaptation period with green tea, a sanitary prevention and prophylaxis program was carried out on the breeding rabbits to control the most common diseases. For this purpose, on the first day, the rabbits were dewormed with 0.1 ml of broad-spectrum Ivermectin injected subcutaneously (Econovet). On the second day and for five days, they were administered Diametoprim (Sanfer) in drinking water at 75 mg kg^{-1} of body weight. From the sixth day, 1 g L^{-1} of Vitafort A (Parfarm) was added to them for three days as a constituent.

2.9. Response variables

Female sexual behavior was assessed by female receptivity to the male. For reproductive behavior, the farrowing rate and the total number of live and weaned young rabbits were evaluated.

The incidence of gel presence (%), seminal volume (ml), and pH were assessed in males. The variables evaluated using the CASA system were total motility, progressive motility, sperm concentration per ml (x106), VSL: rectilinear velocity (µm/s); VCL: curvilinear velocity (µm/s); VAP: average trajectory velocity (µm/s); LIN: linearity index (%); STR: straightness index (%); WOB: oscillation index (%); ALH: mean amplitude of lateral displacement of the sperm head (µm); BCF: cross-hit frequency (Hz); viability (%) and sperm normality (%). For this purpose, at least 400 sperm were counted per sample. Sperm concentration per ejaculate (x106), live, progressive, motile sperm per ejaculate (x106), and the number of doses used in AI were also evaluated.

2.10. Statistical analysis

2.10.1. Statistical analysis for females

Of the 80 that began the experiment, only 71 completed it. The number of patients who completed the study for treatments 1, 2, 3, and 4 was 19, 16, 18, and 18, respectively. The reasons for not completing the experiment were death, technical culling, and pathological causes. Data on dead or discarded does were excluded from the statistical analyses. The sexual receptivity and farrowing rates were considered Bernoulli variables (from 0 to 1). The sexual receptivity rate and farrowing rate were subjected to analysis of variance using the following linear model:

$$\boldsymbol{Y_{ijk} = \mu + H_i + M_j + (H*M)_{ij} + b_1(X_{ijk} - \bar{\bar{X}}) + b_2(X_{ijk} - \bar{\bar{X}}) + b_3(X_{ijk} - \bar{\bar{X}}) + b_4(X_{ijk} - \bar{\bar{X}}) + b_5(X_{ijk} - \bar{\bar{X}}) + \varepsilon_{ijk}}$$

Where: Y_{ijk} is the response variable; μ is the overall mean; H is the fixed effect of the i^{th} green tea extract supplementation in females (i = with, without); M_j is the fixed effect of the j^{th}

green tea supplementation in males (j = with, without)); $(H * M)_{ij}$ is the interaction between green tea supplementation in females and supplementation in males; b_1 is the linear regression coefficient of the covariate insemination session; b_2 is the linear regression coefficient of the covariate age of the rabbit; b_3 is the linear regression coefficient of the covariate season of the year; b_4 is the linear regression coefficient of the covariate weight of the rabbit at insemination; b_5 is the linear regression coefficient of the covariate physiological state at the time of insemination and ε_{ijk} is the residual $\sim NI(0,\sigma^2 e)$.

Litter size at farrowing was analyzed according to a model including the fixed effects of green tea supplementation in females, green tea supplementation in males, the interaction between green tea supplementation in females and green tea supplementation in males, and the covariates: doe age, parity, farrowing order, doe weight at farrowing, and physiological status. The model used for litter size at weaning was the same as for litter size at farrowing and the same covariates, but it included doe weight at weaning.

Covariates that did not show a significant effect ($P > 0.05$) were eliminated from the model, which was then run again.

Response variables related to sexual performance, fertility, litter sizes, and weights were analyzed using the least squares method using the PROC MIXED procedure in SAS [8]. Cumulative productive-reproductive performance variables were analyzed using the least squares method using the GLM procedure in SAS. Means comparisons were performed using Tukey's test.

2.10.1. Statistical analysis for males

The seminal and sperm characteristics, movement, kinematics of sperm movement, viability, morphology, and reproductive potential variables were subjected to analysis of variance considering the following linear additive model:

$$\boldsymbol{Y_{ijk} = \mu + T_i + E_j + (T * E)_{ij} + b_1(X_{ijk} - \bar{\bar{X}}) + b_2(X_{ijk} - \bar{\bar{X}}) + b_3(X_{ijk} - \bar{\bar{X}}) + \varepsilon_{ijk}}$$

Where Y_{ijk} is the response variable; μ is the overall mean; Ti is the fixed effect of the i^{th} treatment (i = without green tea extract, with extract); E_j is the fixed effect of the j^{th} ejaculate (j = first, second); $(R * E)_{ij}$ is the interaction between treatment and ejaculate order; b_1 is the linear regression coefficient of the covariate progressive semen collection session; b_2 is the linear regression coefficient of the covariate season at the time of semen collection; b_3 is the linear regression coefficient of the covariate rabbit weight at the time of semen collection; and ε_{ijkl} is the residual $\sim NI(0,\sigma^2 e)$.

Percentage variables were transformed using arcsine, and sperm concentration per ejaculate was transformed using log 10. All analyses of variance were performed with the original and transformed data. Covariates that did not show a significant effect ($P > 0.05$) were removed from the model, and the model was rerun. The ANOVAs yielded the same response patterns when using the transformed and original data. The ANOVA results are presented with the original data.

Response variables were analyzed using the least squares method using the PROC MIXED procedure in SAS [8]. Means were compared using Tukey's test.

3. Results and discussion

This study continues a line of research focused on supplementation with high-polyphenol green tea extract in New Zealand White rabbits. Previous work, such as that by Mentado [9], evaluated the effects of this supplement in breeding males using a dose of 50 μg/kg of live weight. Subsequently, Miranda [10] deepened this line, increasing the dose to 70 μg/kg and confirming positive effects on various male reproductive parameters. Based on this background, this work aimed to evaluate the impact of the same supplementation but applied to females and analyze possible interactions between males and females under different supplementation combinations. For this purpose, an adjusted dose of 75 μg/kg of live weight was used.

3.1. Females

The rabbits were managed under an AI program with a semi-intensive reproductive rhythm, following a reproductive management approach aimed at maintaining efficient production without compromising the physiological state of the females. This type of reproductive rhythm has been widely used in rabbit breeding due to its balance between productivity and animal welfare, allowing adequate postpartum recovery and efficient synchronization of the reproductive cycle [11,12,13].

Table 1. Descriptive statistics and coefficients of variation for weight and productive-reproductive performance in New Zealand White rabbits.

Parameter	N	Min	Max	Media	SD	CV
Weight of the rabbit at the time of AI (g)	184	3005.0	5080.0	3939.2	425.6	10.8
Age of the rabbits (d)	184	352.0	633.0	5’06.6	82.1	16.2
Sexual receptivity rate	184	0.0	1.0	0.6	0.5	78.6
Birth rate	184	0.0	1.0	0.7	0.5	67.2
Weight of the rabbit at birth (g)	127	3170.0	4760.0	3936.3	375.3	9.5
Total babies born	127	1.0	13.0	7.2	2.7	37.8
Live-born rabbits	127	0.0	13.0	6.9	2.9	42.1
Weaned young rabbits	127	0.0	10.0	5.6	2.8	49.7
Total weight of rabbits born (g)	127	60.0	950.0	423.8	156.6	36.7
Weights of live-born rabbits (g)	127	0.0	950.0	408.6	167.4	41.0
Weights of weaned young rabbits (g)	127	0.0	6875.0	3713.0	1709.1	46.0
Total individual birth weights	127	23.5	112.5	61.7	14.5	23.4
Individual live birth weights (g)	123	23.5	112.5	61.9	13.7	22.1
Individual weaned weights (g)	112	420.0	1127.7	688.9	134,4	19.5

SD= Standard deviation; CV= Coefficient of variation (%)

The variables with the least dispersion were the weight and age of the does, indicating that the experimental group consisted of animals with similar physiological characteristics regarding maturity and body condition. In contrast, the variables with the most excellent dispersion were the sexual receptivity rate and farrowing rate because these are binary variables (0-1), where minor individual differences have a greater impact on the overall variation. Similarly, the number of live-born and weaned young rabbits showed high variability, which can be attributed to physiological, hormonal, and behavioral differences between the females and to the uterus condition, semen quality, and maternal skills.

The individual weights of the young rabbits at birth (61.7 g) and weaning (688.9 g) were more homogeneous than the total litter weights, suggesting that individual development was relatively constant regardless of litter size.

The wide variability observed in rabbit reproductive parameters is common in AI studies, especially under experimental conditions [12]. High coefficients of variation in variables such as receptivity rate and parturition reflect physiological and hormonal differences between animals.

Regarding litter size, the mean total number (7.2) and live births (6.9) are similar to those reported by El-Ratel et al. [1], who observed identical values in rabbits supplemented with natural extracts. Individual weights (61.7–61.9 g total and live births, respectively) are within the optimal range reported by Fraga et al. [14], who associated these values with improved viability and lower pre-weaning mortality.

On the other hand, total litter weight at weaning (3713 g) and individual weight (688.9 g) are positive indicators of postnatal growth. However, the high dispersion significantly influences maternal behavior, milk access, and milk production differences. This variability is consistent with that reported by Méndez et al. [15], who highlighted the influence of physiological and environmental factors on lactation success.

The effects of green tea extract supplementation on female and male rabbits on sexual receptivity and parturition rate are presented in Table 2. The covariates of doe age (p=0.010), season (p=0.013), and physiological state at the time of insemination (p=0.004) were significant for receptivity, so these variables were adjusted with the following standard values: 506.5 days, 1.54, and 2.50, respectively. In the case of the farrowing rate, the significant covariates were season (P = 0.0001), parity (P = 0.050), farrowing order (P = 0.0001), and physiological state (P = 0.008) being adjusted with the following standard values respectively 1.54, 2.80, 0.995 and 2.50.

Supplementation in females had a significant effect on sexual receptivity (P = 0.003), with a higher mean age in supplemented does (0.72 ± 0.05) compared to unsupplemented does (0.52 ± 0.05), representing a 38.5% improvement. However, no significant differences were found in the farrowing rate (P = 0.757).

Regarding males, no significant effects were observed on either sexual receptivity (P = 0.210) or farrowing rate (P = 0.574). However, interactions between factors showed a trend toward higher receptivity values in the combined groups with supplementation in females: 1H1M (0.74 ± 0.07) and 1H2M (0.71 ± 0.07). In contrast, the lowest value was present in the group without supplementation (2H*2M: 0.44 ± 0.06). No significant differences were found in the interaction for the farrowing rate (P = 0.831).

Regarding sexual receptivity, a significant interaction was evident between green tea extract supplementation in males and females (p = 0.003). Supplemented females showed greater receptivity when supplemented males were used (0.72) than when mated with non-supplemented males (0.67). This difference was even more pronounced in non-supplemented females, where receptivity decreased from 0.58 to 0.52 depending on whether the male had received the supplement. The absence of parallelism in the lines of the graph confirms the existence of an interaction, suggesting that the supplement's effect varies according to the sex to which it is administered (Figure 2). In contrast, the parturition rate did not show significant differences between treatments (p = 0.831), showing practically parallel graphic lines. The values remained stable in supplemented females (0.69). They barely varied in non-supplemented females (0.68 vs. 0.69), indicating that supplementation, in both males and females, did not have a relevant impact on this reproductive parameter (Figure 3).

The results show that green tea extract supplementation in females significantly improved sexual receptivity, which could be related to the antioxidant effect of green tea compounds on the reproductive system [16]. The 38.5% improvement in receptivity indicates that the supplement favors the response to the biostimulus and possibly the expression of estrus, as has been reported in other studies with natural antioxidants [17,18,19].

Table 2. Least squares mean (± standard error) for the effect of green tea supplementation on females and males and interactions on sexual receptivity and parturition rate in rabbits.

Factor	N	Sexual receptivity[1]	calving rate[2]
Green tea in females (H)			
With (1)	91	0.72 ± 0.05[a]	0.69 ± 0.013
Without (2)	93	0.52 ± 0.05[b]	0.69 ± 0.013
Valor - *P*		0.003	0.757
Green tea in males (M)			
With (1)	93	0.67 ± 0.05	0.69 ± 0.013
Without (2)	91	0.58 ± 0.05	0.68 ± 0.013
Value - P		0.210	0.574
Interaction between factors			
1 H * 1 M	47	0.74 ± 0.07	0.69 ± 0.018
1 H * 2 M	44	0.71 ± 0.07	0.68 ± 0.019
2 H * 1 M	46	0.59 ± 0.07	0.70 ± 0.018
2 H * 2 M	47	0.44 ± 0.06	0.68 ± 0.018
Value - P		0.389	0.831

1. Rabbit age, season, and physiological status at the time of artificial insemination were used as covariates.
2. Season and physiological status were used as covariates.

Means for the same factor with different letters were different ($P < 0.003$).

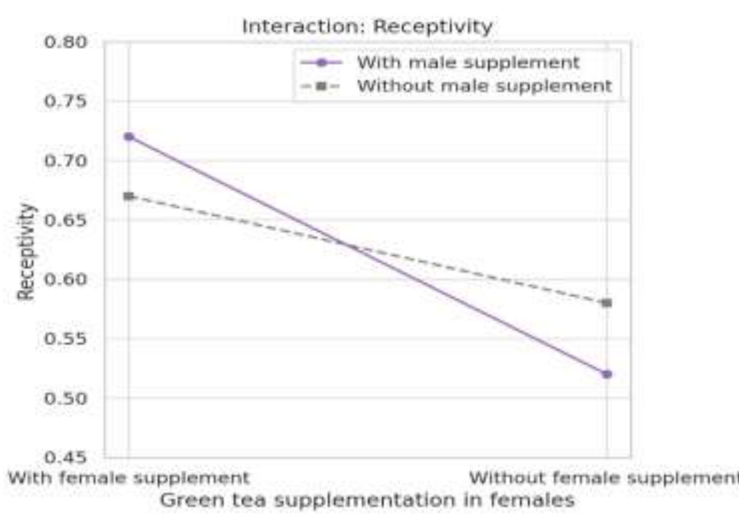

Figure 2.Interaction between supplementation in males and females on receptivity.

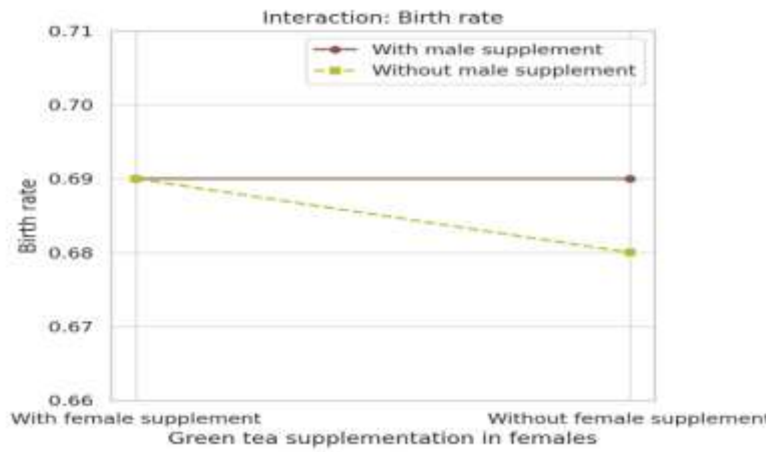

Figure 3.Interaction between supplementation in males and females on birth rate.

The absence of significant effects on parturition rate suggests that, although receptivity is enhanced, this does not necessarily translate into a direct improvement in conception rate or pregnancy maintenance. This is consistent with the report by Hashem et al. [20]. who observed that antioxidants can improve certain reproductive aspects without directly impacting final fertility.

Although supplementation in males did not show significant differences, a slight favorable trend in receptivity was observed when at least the females were supplemented, especially in the 1H1M and 1H2M groups, which could indicate a synergistic or at least complementary effect of the treatment. The unsupplemented group (2H*2M) showed the lowest receptivity, reinforcing the potential usefulness of green tea as a reproductive aid.

Significant covariates also provide valuable information: age, season, and physiological state influenced receptivity, consistent with previous studies [21] highlighting that animals in better physiological condition and reproductively favorable seasons responded better to the stimulus. In the case of farrowing rate, season, parity, farrowing order, and physiological state were significant, highlighting the complexity of this parameter and the need to control these variables to achieve sustained improvements. Identifying the physiological and management factors that influence these variables is essential for optimizing reproductive performance in AI programs in rabbits.

The effects of green tea extract on litter sizes, expressed as total births, live births, and weaned numbers, are shown in Table 3. The covariate farrowing order was significant for total births (p=0.031), live births (p=0.003), and weaned numbers per farrowing (p=0.004), so this variable was adjusted to a standard value of 1.43. For weaned numbers per farrowing, the significant covariates were farrowing order (p=0.004), doe birth weight (p=0.011), and doe weaning weight (p=0.0001), which were adjusted to the following standard values: 1.43, 3904.0, and 3645.5, respectively. Supplementation in females significantly affected the total number of live births (P = 0.005) and live births (P = 0.014). These effects were higher in supplemented does (7.84 ± 0.32 and 7.47 ± 0.34) compared to non-supplemented does (6.54 ± 0.33 and 6.26 ± 0.34), representing an increase of 19.9% and 19.3%, respectively. No significant differences were observed for the number of weaned rabbits (P = 0.095), although there was a slight trend in favor of the supplemented group.

No significant effects were observed in males on any of the variables evaluated (P > 0.05). Regarding interactions, there were no significant effects, but the 1H2M and 1H1M groups presented the highest values of live births (8.27 ± 0.47 and 7.41 ± 0.44) and weaned (6.34 ± 0.37 and 5.51 ± 0.36), suggesting a possible synergy in cross-supplementation.

Table 3. Least squares mean (± standard error) for the effect of green tea extract supplementation on both females and males and interactions on litter sizes at farrowing and weaning in New Zealand White rabbits

Factor	N	Total born[1]	Live born[1]	Weaned[2]
Green Tea in Females (H)				
With (1)	64	7.84 ± 0.32[a]	7.47 ± 0.34[a]	5.93 ± 0.256
Without (2)	63	6.54 ± 0.33[b]	6.26 ± 0.34[b]	5.32 ± 0.258
P-Value		0.005	0.014	0.095
Green Tea in Males (M)				
With (1)	64	7.16 ± 0.32	6.94 ± 0.34	5.37 ± 0.26
Without (2)	63	7.22 ± 0.32	6.80 ± 0.34	5.88 ± 0.26
P-Value		0.894	0.774	0.170
Interaction between factors				
1 H * 1 M	34	7.41 ± 0.44	7.15 ± 0.47	5.51 ± 0.36
1 H * 2 M	30	8.27 ± 0.47	7.80 ± 0.50	6.34 ± 0.37
2 H * 1 M	30	6.90 ± 0.47	6.73 ± 0.50	5.23 ± 0.38
2 H * 2 M	33	6.17 ± 0.45	5.80 ± 0.48	5.41 ± 0.36
P-Value		0.084	0.107	0.379

1. Farrowing order used as a covariate.
2. Farrowing order, doe weight at farrowing, and doe weaning weight used as covariates.

Means on the same factor with different letters are different ($p<0.005$; $p<0.01$).

Figures 4, 5, and 6 show a consistent trend in green tea extract supplementation in females (F) and males (M). For all three reproductive variables evaluated, the F+M− group (females supplemented, but not males) had the highest values: 8.27 total births, 7.80 live births, and 6.30 weaned, suggesting a predominantly positive effect of maternal supplementation. In contrast, the F−M− group (no supplementation in either sex) showed the lowest performance across all categories, while the F+M+ and F−M+ groups had intermediate values. These results indicate that supplementation in females has a greater impact on reproductive performance and that additional supplementation in males does not appear to enhance the benefits when females are already supplemented.

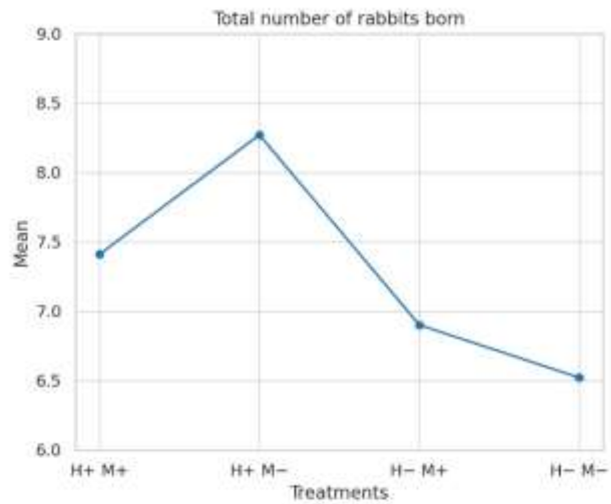

Figure 6.Treatment trend in the total number of rabbits born.

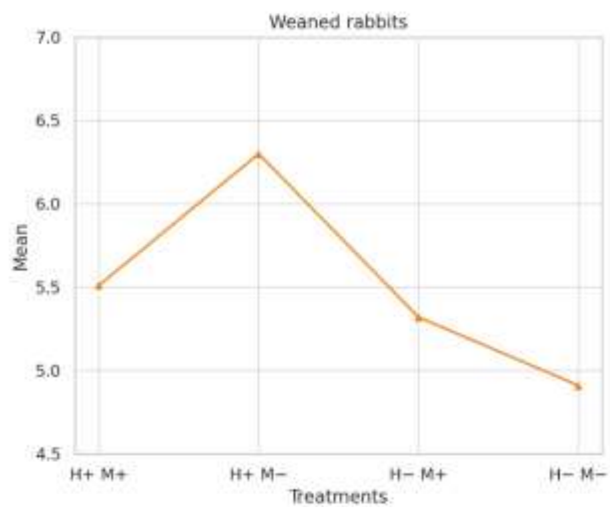

Figure 5.Treatment trend in the total number of weaned rabbits.

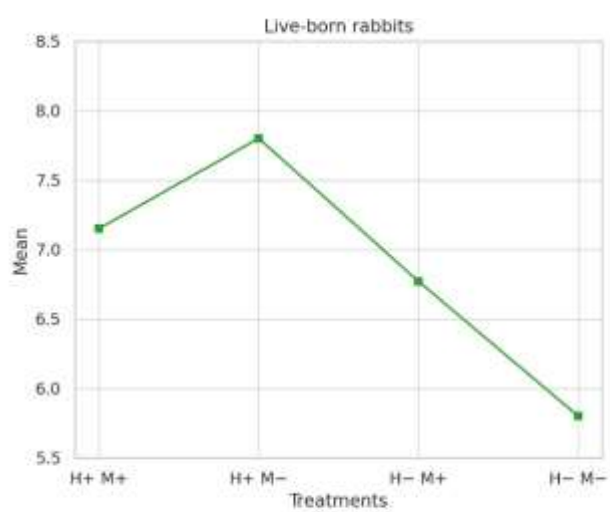

Figure 4.Treatment trend in the total number of live-born rabbits

Green tea extract supplementation in female rabbits significantly improved litter size at parturition in terms of the number of total and live-born young rabbits. This is consistent with the report by El-Ratel et al. [1], who found that this extract promotes ovarian activity, possibly due to its antioxidant, anti-inflammatory, and hormone-regulating effects.

In the present study, the average number of live young rabbits born per delivery was 7.47 in the group of does supplemented with green tea extract, while in the group without supplementation, it was 6.26. These results are similar to those reported by those who observed averages of 5.6 live young rabbits per delivery, respectively, in rabbits fed under standard conditions [22]. In the case of weaned young rabbits, the average in the supplemented group was 5.93, compared to 5.32 in the group without supplementation. These coincidences reinforce the data's consistency and support green tea's efficacy as a functional additive in rabbit reproduction. Furthermore, other authors, such as Pascual et al. [23], reported averages of 7.1 to 7.5 live births per parity in feed-enriched diets, which is consistent with the values observed in this study. In the case of weaned rabbits, reported values close to 6.0 offspring in well-managed reproductive programs, which also aligns with the findings of the present experiment [24]. Thus, natural extracts such as green tea can be considered a viable alternative to promote prolificacy parameters under controlled conditions.

The lack of a significant effect in males may be attributed to the fact that the beneficial effects of green tea are more evident in semen parameters than in direct fertilization capacity [20]. However, the observation of higher values in the groups with supplemented females reinforces the importance of maternal physiological status in determining litter size.

Regarding covariates, calving order was decisive in all the parameters evaluated, as reproductive experience significantly influences the response to nutritional treatments. Likewise, the weight of the rabbit at birth and weaning was a determining factor, in line with studies demonstrating its association with reproductive efficiency and suckling success [15].

Although the number of weaned rabbits was not significantly different in the supplemented groups, a positive trend was observed. This suggests that improving uterine and embryonic conditions could extend to postnatal development. This effect could depend on other factors such as milk production, maternal behavior, or initial litter size [11].

Table 4 presents the effects of green tea extract on total litter weight at birth (total born and live born) and at weaning. The covariate parturition order was significant for total litter weight ($p=0.0003$), live-born litter weight ($p=0.0001$), and weaning litter weight ($p=0.0001$) and was adjusted to a standard value of 1.43. Another significant variable for total litter weight was doe age ($p=0.032$), which was adjusted to a standard value of 501.3. On the other hand, the variables parity ($p=0.0001$) and doe weight at weaning ($p=0.0001$) were also significant for litter weight at weaning, adjusted to the following standard values: 2.81 and 3645.5, respectively.

Supplementation in females significantly affected total litter weight at weaning ($P=0.032$), with an average value of 3929.5 g versus 3499.2 g in unsupplemented females, representing a 12.3% increase. No significant differences were found for total litter weight at birth ($P>0.05$).

In males, a significant effect on total weaning weight was also observed ($P = 0.032$), where unsupplemented animals had a higher weight (3930.6 g) compared to the supplemented ones (3498.2 g), representing a difference of 12.3% against the treated group. The interactions were not significant for any of the variables ($P > 0.05$), although the 1H2M and 2H2M groups had the highest average weaning weights (4170.3 ± 202.4 g and 3690.8 ± 194.8 g, respectively).

Table 4. Least squares mean (± standard error) for the effect of green tea extract supplementation on females and males and interactions on total litter weights at farrowing and weaning in New Zealand White does.

Factor	N	Litter weights Total born[1]	Litter weights born alive[2]	Litter weaning weights[3]
Green tea in females (H)				
with (1)	64	437.4 ± 17.8	420.7 ± 19.2	3929.5 ± 139.4[a]
without (2)	63	411.1 ± 18.7	397.4 ± 19.4	3499.2 ± 140.5[b]
Value - P		0.299	0.395	0.032
Green tea in males(M)				
with(1)	64	436.5 ± 18.1	428.6 ± 19.2	3498.2 ± 139.6[b]
without (2)	63	411.9 ± 18.3	389.6 ± 19.4	3930.6 ± 140.5[a]
Value - P		0.349	0.156	0.032
Interaction between factors				
1 H * 1 M	34	438.7 ± 25.5	427.3 ± 26.3	3688.1 ± 191.4
1 H * 2 M	30	436.1 ± 26.1	414.2 ± 28.0	4170.3 ± 202.4
2 H * 1 M	30	434.4 ± 26.3	429.9 ± 28.1	3307.6 ± 204.1
2 H * 2 M	33	387.7 ± 25.2	365.0 ± 26.7	3690.8 ± 194.8
Value - P		0.385	0.345	0.804

[1]. Rabbit age and birth order used as covariates.
[2]. Birthing order used as covariate.
[3]. Parity, birth order, and weaning weight of the rabbit used as covariates.
Means on the same factor with different letters are different (P < 0.032).

Several studies have reported proper reference values to contextualize the results obtained here. For example, Rommers et al. [25] reported litter birth weights of 420 and 460 g in does, figures similar to those observed in the present study. Similarly, documented averages of 440 to 470 g in live births per litter in breeding under standard management. In contrast, the results obtained are higher than those reported by Meo et al. [26] and Zerroki et al. [27], who reported 270 and 248 g litter weights, respectively. Birth weight is influenced by the birth order, with primiparous producing lower weights than multiparous [27]. Regarding the total litter weight at weaning, Theau-Clément et al. [11]. and Xiccato et al. [24] found ranges between 3500 and 3900 g depending on the number of young rabbits and the quality of postnatal management. These results are consistent with the averages obtained in this study, especially in the groups supplemented with green tea extract, which showed values close to 3930 g.

In this study, total litter weight at weaning was significantly higher in those supplemented with green tea extract. This 12.3% improvement can be attributed to improved maternal physiological and nutritional conditions influenced by green tea's antioxidant effects.

Although total litter weights at birth did not show statistical differences, the observed means (437.4 g vs. 411.1 g in does with and without supplementation, respectively) suggest a slight effect, consistent with the report by Gutiérrez et al. [28], who noted that the impact of supplements such as prebiotics, probiotics, and plant extracts is usually more evident in the postnatal stages than in birth weight. The adverse effect observed in supplemented males could be related to alterations in the seminal microbiota or non-beneficial physiological interactions, as suggested by Hashem et al. [20], who observed mixed effects of antioxidants such as selenium, vitamin C, and rosemary extract on offspring depending on the route of administration and the reproductive stage of the male.

The effects of green tea extract on individual rabbit weights at birth (total and live births) and weaning are shown in Table 5. The covariate parturition order was adjusted to a value of 1.43 as it was significant for the variables total individual birth weight ($p=0.023$), individual live birth weight ($p=0.023$), and individual weaning weight ($p=0.055$). Likewise, the covariate doe weight at parturition was adjusted to a value of 3904.0 as it was significant for the variables total individual birth weight ($p=0.0001$) and individual live birth weight ($p=0.0001$). Finally, the covariate doe weight at weaning ($p=0.015$) was significant for individual weaning weights, being adjusted to a value of 3645.5.

Significant differences favoring the non-supplemented group were observed in females for individual birth weights of total ($P = 0.011$) and live births ($P = 0.004$), with values of 64.5 ± 1.56 g and 64.8 ± 1.51 g, respectively, compared with 58.8 ± 1.55 g and 58.6 ± 1.53 g in the supplemented females. No significant differences were observed in weaning weights ($P = 0.688$), although values were slightly higher in the unsupplemented females.

No significant differences were observed in any of the three parameters in males, although higher live birth values (63.9 ± 1.52 g) were reported in supplemented males compared to unsupplemented males (60.9 ± 1.53 g). The interactions were also insignificant, although the 2H*1M group showed the highest individual weaning weight (7.241 ± 2.50 g).

Individual birth and weaning weights were slightly higher in the groups supplemented with green tea extract. However, no statistically significant differences were found, except for the weight of live-born rabbits compared to unsupplemented males. This trend may indicate a slight effect of supplementation on fetal and postnatal development since, under controlled conditions, variability in individual weight depends largely on litter size and the mother's milking capacity rather than on diet per se [29].

Xiccato et al. [24] reported individual weaning weights of between 650 and 710 g in rabbits under intensive reproductive management, which is consistent with the values observed in this study. In contrast, Zerrouki [27], reported weaning weights of 475 g, which is lower than the results obtained.

In the literature, several authors have addressed the influence of physiological and reproductive factors on the individual development of young rabbits. For example, Pascual et al. [23] observed that those with better body conditions produce more homogeneous litters with higher individual weights. These findings were reinforced by Castellini et al. [12], who demonstrated that reproductive efficiency is closely related to the mother's physical condition during gestation and lactation.

Finally, in this study, farrowing order and number of live births were key covariates influencing the results, as smaller litters tend to have heavier offspring, both at birth and weaning, due to less competition for intrauterine nutrients and breast milk.

Table 6 presents the descriptive statistics for the variables accumulated during the experimental period. The average number of inseminations per dose was 2.6, with a CV of 23.1%. The number of births averaged 1.8, with a CV of 22.2%, consistent with the observed farrowing rate (74.3%), an acceptable value in AI systems.

Table 5. Least squares mean (± standard error) for the effect of green tea extract supplementation on females and males and interactions on individual litter weights at farrowing and weaning in New Zealand White does.

Factor	N	Total Individual Birth Weights[1]	Individual weights Live births[1]	Individual Weaned Weights[2]
Green tea in females(H)				
with(1)	64	58.8 ± 1.55[b]	58.6 ± 1.53[b]	684.4 ± 17.3
without(2)	63	64.5 ± 1.56[a]	64.9 ± 1.51[a]	693.9 ± 17.3
P-value		0.011	0.004	0.698
Green tea in males(M)				
with(1)	64	64.5 ± 1.56[a]	63.9 ± 1.52[a]	681.6 ± 17.1
without(2)	63	58.8 ± 1.60[b]	59.6 ± 1.53[b]	696.7 ± 17.7
P-value		0.012	0.049	0.543
Interaction between factors				
1 H * 1 M	34	62.6 ± 2.15	61.4 ± 2.12	669.4 ± 24.4
1 H * 2 M	30	55.1 ± 2.28	55.8 ± 2.23	669.4 ± 25.2
2 H * 1 M	30	66.4 ± 2.26	66.4 ± 2.18	663.7 ± 24.1
2 H * 2 M	33	62.6 ± 2.16	63.5 ± 2.11	724.1 ± 25.0
P-value		0.406	0.539	0.073

[1]. Birthing order and doe birth weight used as covariates.
[2]. Birthing order and doe weaning weight used as covariates.
Means on the same factor with different letters are different ($p < 0.01$; $p < 0.04$).

The number of weaned litters averaged 1.6, while the interval between farrowing and insemination was 14.9 days, slightly higher than the optimal interval of 10 to 12 days, as cited by Theau-Clément [11]. The total and live births averaged 13.2 and 12.3 young rabbits, respectively, with moderate variations indicating some dispersion among females. The average number of weaned young rabbits was 9.2, representing 74.8% of the total number born.

The average weights per total, live birth, and weaned young rabbit were 0.7, 0.7, and 6.5, respectively. The latter was the most variable, reflecting differences in maternal ability and competition between young.

The cumulative results of this study allow for comparisons with various authors who have reported reproductive parameters under AI programs in rabbits. In this regard, Rebollar [30] indicated that the average number of inseminations required per birth can vary between 1.5 and 2.5 depending on the hormonal protocol used and the efficiency of the biostimulant, which supports the values observed in this study.

Table 6. Descriptive statistics and coefficients of variation for the accumulated productive-reproductive performance during the experimental period in New Zealand White rabbits.

Variable	N	Min	Max	Media	SD	CV
Number of inseminations	71	2.0	4.0	2.6	0.6	23.1
Number of births	71	1.0	2.0	1.8	0.4	22.2
Inseminations/births	71	1.0	4.0	1.6	0.8	47.4
Farrowing rate	71	25.0	100.0	74.3	25.5	34.4
Number of weaned litters	71	1.0	2.0	1.6	0.5	31.1
Farrowing interval between inseminations (d)	71	11.0	28.3	14.9	5.0	33.6
Total births	71	5.0	21.0	12.8	4.7	36.4
Live births	71	4.0	21.0	12.3	4.9	39.5
Weaned births	71	2.0	21.0	10.2	4.2	41.2
Total weight of born young rabbits (g)	71	0.3	1.5	0.7	0.3	37.3
Live birth weights (g)	71	0.3	1.5	0.7	0.3	38.8
Weaned young rabbits	71	1.9	12.0	6.5	2.3	36.3

SD= Standard deviation; CV= Coefficient of variation (%)

On the other hand, Garreau et al. [31] reported farrowing rates between 65 and 75% in rabbits under controlled photoperiods, which coincides with the average of 74.3% recorded in this experiment.

Furthermore, Lukefahr et al. [32] emphasized that cumulative weaning weights reflect reproductive efficiency and maternal ability. Their study reported average cumulative weights between 6.0 and 6.7 kg per weaned litter, comparable to the average of 6.5 kg observed in this study.

The average of 2.6 inseminations per dose is slightly higher than that of Gutiérrez et al. [28], who documented averages between 2.1 and 2.4 in well-structured AI protocols. This could be related to individual variability in response to the biostimulus and the estrus detection efficiency [11].

The average of 13.2 total accumulated young rabbits born and 12.3 live rabbits born per doe during the experimental period aligns with the values reported by Pascual [23], who established ranges of 12–14 accumulated young per complete reproductive cycle in intensive systems. The weaning rate (9.2), considering the influence of factors such as maternal behavior and pre-weaning mortality.

Various productive and reproductive indicators accumulated during the experiment were evaluated based on the treatments applied, which are shown in Table 7. Although most variables did not show significant differences ($P > 0.05$), essential trends were identified. The total number of births was significantly higher ($P < 0.02$) in treatment T2 (15.6 ± 1.1), followed by T1 (13.3 ± 1.02), compared to T3 and T4 (11.4 ± 1.05 and 11.3 ± 1.05, respectively), suggesting a favorable effect of supplementation in females compared to unsupplemented males (T2). For live births, although statistical significance was not reached ($P = 0.064$), a clear favorable trend was observed in treatments T1 and T2 (12.9 ± 1.08 and 14.7 ± 1.18) over T3 and T4 (11.2 ± 1.1 and 10.7 ± 1.1), with a relative difference of up to 27.3% in favor of the T2 group versus the T4 group. The same occurred with weaned infants, where T2 had the highest value (11.9 ± 1.04), followed by T1 (10.0 ± 0.96), although not statistically significant ($P = 0.288$). Regarding the average weights of young rabbits (total born, live-born, and weaned), no significant differences were found either (P > 0.05), although the T2 treatment maintained the highest values in all indicators: 0.815 g in total born, 0.792 g in live born and 7.728 g in weaned.

Table 7. Least squares mean (± standard error) for the treatment effect on cumulative productive-reproductive performance over the experimental period.

Variable	T1 (19)	T2 (16)	T3 (18)	T4 (18)	Value - P
Number of inseminations	2.47 ± 0.14	2.75 ± 0.15	2.55 ± 0.14	2.61 ± 0.14	0.595
Number of births	1.79 ± 0.09	1.94 ± 0.09	1.67 ± 0.09	1.83 ± 0.09	0.263
Inseminations/birth	1.52 ± 0.17	1.47 ± 0.19	1.78 ± 0.18	1.56 ± 0.18	0.643
Births/inseminations	0.76 ± 0.06	0.74 ± 0.06	0.70 ± 0.06	0.76 ± 0.06	0.886
Birth-insemination interval (d)	14.5 ± 1.69	14.8 ± 1.27	15.0 ± 1.20	15.1 ± 1.20	0.982
Total births	13.3 ± 1.02^{ab}	15.6 ± 1.11^{a}	11.4± 1.05^{b}	11.3 ± 1.05^{b}	0.025
Live births	12.9 ± 1.08	14.7 ± 1.18	11.2 ± 1.11	10.7±1.11	0.064
Weaned births	10.0± 0.96	11.9 ± 1.04	9.5 ± 0.98	9.4 ± 0.98	0.288
Total birth weights (g)	0.790 ± 0.06	0.815 ± 0.07	0.731 ± 0.06	0.663 ± 0.06	0.387
Live birth weights (g)	0.742 ± 0.06	0.792 ± 0.07	0.707 ± 0.07	0.654 ± 0.07	0.489
Weaned weights (g)	6.283 ± 0.53	7.728 ± 0.57	5.997 ± 0.54	6.050 ± 0.54	0.109

Means on the same factor with different letters are different (P < 0.02).

The results in Table 7 are based on the analysis of the treatment on the accumulated reproductive performance per rabbit during the experimental period. T2 was significantly higher in the total number of born (P < 0.02) and showed the best means in most parameters, even without statistical significance. These findings are consistent with those reported by El-Ratel et al. [1], who found that green tea extract improved fertility and stimulated ovulation in rabbits.

Improved uterine conditions, derived from nutrition, influence embryo implantation, which could explain the higher number of live births observed at T2. Larger litters could be associated with a higher ovulation rate and better receptivity conditions. Although no significant differences were found in individual weights, the higher value observed at T2 in all weight indicators (total, live births, and weaned) suggests a beneficial effect on fetal and postnatal development. These increases could derive from higher milk production or lower intrauterine competition. Notably, the T4 treatment, without supplementation, showed the lowest values for most variables. This pattern suggests that reproductive efficiency could be compromised without nutritional intervention. The constant improvement at T2 reinforces the hypothesis that the effects of green tea on the reproductive female could be more decisive than on the male [20].

Although only the total number of births reached statistical significance, the clear trend favoring T2 in almost all the parameters evaluated supports its potential as a reproductive improvement strategy. These results reinforce the need to assess sex-differentiated treatments and to continue studies that integrate both reproductive and economic performance.

Among the treatments evaluated, significant differences were observed in the reproductive variables of total births (P = 0.018), as well as trends in live birth (P = 0.064) and weaning (P = 0.288). Treatment T2, corresponding to does supplemented with green tea extract but mated with non-supplemented males, showed the best results, with averages of 15.6 total offspring born, 14.7 live births, and 11.9 weanings per female. In comparison, the control treatment (T4), in which neither females nor males received supplements, showed the lowest values for these variables (11.3, 11.7, and 9.4, respectively). These results suggest that green tea supplementation in females positively affects productive and reproductive performance, especially when no supplementation is applied to males. This could be due to an improvement in the uterine environment, antioxidant status, or reproductive efficiency of the rabbits (Figure 7).

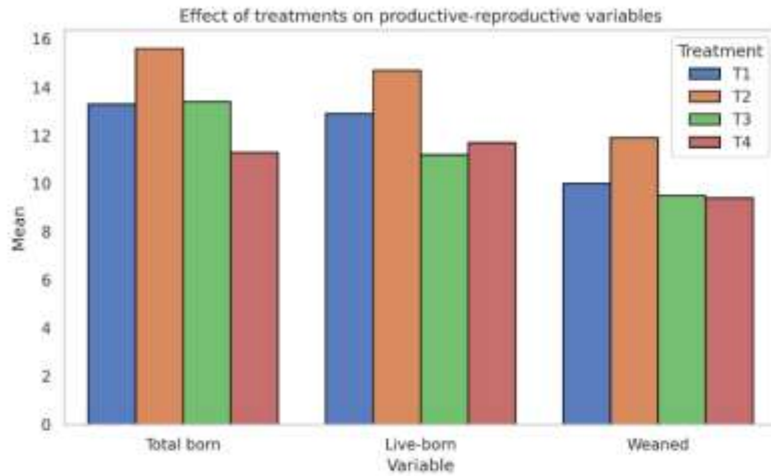

Figure 7.effect of treatments on productive-reproductive variables.

3.2. *Males*

The rabbits were managed under an extensive breeding regimen, which consisted of collecting the first and second ejaculate once a week. This type of breeding regimen has been shown to favor the production of ejaculates with greater volume and concentration compared to semi-intensive regimens, where two ejaculates are collected twice a week per session. These differences have been linked to a longer recovery time for accessory glands, as well as complete maturation of stored sperm [33,34,35,36,37]

A total of 153 samples from the eight male rabbits used for AI were statistically analyzed. Tables 8 and 9 present descriptive statistics for rabbit weight at the time of semen collection, semen characteristics, sperm characteristics, motility, viability, normality, sperm movement kinematics, and concentration of live, normal, progressive, motile sperm and potential doses for use in AI in rabbits.

The covariate, male weight at the time of semen collection, was observed to significantly influence ($p < 0.05$) 10 of the 26 variables evaluated: semen volume, sperm concentration per ejaculate, progressive motility, straight-line velocity (SLV), average trajectory velocity (ATV), linearity index (LIN), straightness index (STR), wobble index (WOB), mean amplitude of lateral displacement of the sperm head (ALH), and cross-strike frequency (BCF). These last variables were adjusted to a standard weight of 4205.4 g. The covariate semen collection session showed a significant effect ($p < 0.05$) on five variables: sperm concentration per ejaculate, progressive motility, number of live, normal, progressively motile sperm per ejaculate, number of potential doses for AI, and percentage of normal sperm, adjusted for a standard session of 11.38.

The effects of green tea supplementation on semen characteristics and sperm concentration per ejaculation are shown in Table 10. Significant effects were observed in the presence of gel ($P = 0.004$), sperm volume ($P = 0.0001$), and sperm concentration ($P = 0.0003$). Supplemented males had 130.8% more gel, 33.9% more volume, and 149.1 million more sperm per ejaculation than unsupplemented males.

Table 8. Descriptive statistics and coefficients of variation for male weight, semen quality, motility and sperm movement kinematics in New Zealand White rabbits.

Variable	N	Min	Max	Media	SD	CV
Rabbit weight (kg)	153	3230.0	4840.0	4199.9	369.0	8.5
Semen characteristics						
Presence of gel (%)	153	0.0	1.0	0.2	0.4	191.3
Volume (mL)	153	0.2	2.2	0.7	0.4	53.9
pH	153	7.0	8.0	7.0	0.1	1.6
Sperm characteristics Sperm						
concentration/ejaculate(x 10^6)	153	13.6	1446.9	282.6	286.6	100.1
Sperm viability (%)	153	85.0	100.0	97.3	2.3	2.4
Motility and kinematics of sperm movement						
Total motility (%)	153	56.4	98.8	89.2	7.1	7.9
Progressive motility (%)	153	8.8	75.4	46.8	16.2	34.7
VSL (µm/sec)	153	35.3	126.9	73.9	19.7	26.7
VCL (µm/sec)	153	123.3	260.9	181.0	28.8	15.9
VAP (µm/sec)	153	58.7	140.2	99.3	21.3	21.5
LIN (%)	153	24.7	69.3	42.4	8.29	21.1
STR (%)	153	49.0	92.4	74.3	10.0	13.5
WOB (%)	153	41.6	73.8	55.0	5.9	10.7
ALH (mm)	153	4.4	11.4	7.5	1.4	18.2
BCF (HZ)	153	27.7	46.1	36.2	3.5	9.6
DSL (microns)	153	13.9	55.5	29.3	9.5	32.5
DCL (microns)	153	48.9	100.1	74.2	11.4	15.3
DAP (microns)	153	21.8	64.4	39.9	8.9	22.2

SD= Standard deviation; CV= Coefficient of variation (%); VSL: Straight line velocity (µm/s); VCL: Curvilinear velocity (µm/s); VAP: Average trajectory velocity (µm/s); LIN: Linearity index (%); STR: Straightness index (%); WOB: Oscillation index (%); ALH: Mean amplitude of lateral displacement of the sperm head (µm); BCF: Cross-hit frequency (Hz); DSL: Straight line distance (Microns); DCL: Curvilinear distance (Microns); DAP: Average trajectory distance (Microns).

Table 9. Descriptive statistics and coefficients of variation for sperm normality, morphology, concentration of live, normal, progressively motile sperm per ejaculate, and potential doses for artificial insemination in New Zealand B rabbits.

Variable	N	Min	Max	Media	SD	CV
Normal sperm (%)	153	51.7	100.0	92.0	7.7	8.3
Sperm viability	153	85.0	100.0	97.3	2.3	2.4
Sperm with bent tail(%)	153	0.2	12.0	2.9	1.9	65.8
Sperm with coiled tail (%)	153	0.0	7.1	1.5	1.3	84.9
Sperm with distal droplet(%)	153	0.7	44.7	8.2	7.4	91.1
Sperm with proximal droplet (%)	153	0.0	3.6	0.6	0.5	93.8
Number of live, motile, progressive sperm/ejaculate (x10^6)	153	4.0	671.9	110.3	116.5	105.6
Number of doses for use in artificial insemination	153	0.8	134.4	22.1	23.3	105.6

SD= Standard deviation; CV= Coefficient of variation (%)

Regarding the ejaculation order, the first ejaculate showed significantly higher values in the presence of gel (P = 0.0001), sperm volume (P = 0.0001), and sperm concentration (P = 0.0004) compared to the second, with increases of 350%, 97.8%, and 68.3%, respectively. Adjustments were made for the season (P = 0.007) and male weight at collection (P = 0.044) in seminal volume with a value of 1.68 and 4205.4, respectively, as well as the season (P = 0.0001) for sperm concentration to a value of 1.68.

Table 10. Least squares mean (± standard error) for the effects of green tea supplementation and ejaculate order on seminal characteristics and sperm production in New Zealand White rabbits.

	Suplementation			Ejaculate order		
	Without Green Tea N = 83	With Green Tea N = 70	P-value	First N = 79	Second N = 74	P-value
Semen characteristics						
Presence of gel(%)	0.13 ± 0.04^b	0.30 ± 0.04^a	0.004	0.36 ± 0.04^a	0.08 ± 0.04^b	0.0001
Volume (mL) * °	0.59 ± 0.02^b	0.79 ± 0.02^a	0.0001	0.91 ± 0.02^a	0.46 ± 0.02^b	0.0001
pH	7.0 ± 0.01	7.0 ± 0.01	0.196	7.0 ± 0.01	7.0 ± 0.02	0.963
Sperm count/ejaculate (x10^6) *	211.8^a ± 27.0	360.9 ± 29.3	0.0003	359.2 ± 27.7^a	213.4 ± 28.6^b	0.0004

* - The season at the time of semen collection used as a covariate.
° - Doe weight at the time of semen collection used as a covariate.
abc - Letters with different superscripts in the same row differ (P < 0.05).

Green tea extract supplementation significantly improved semen quality, which is consistent with the reports of Hashem et al. [20] and El-Ratel et al. [1], who described that green tea polyphenols promote membrane integrity and increase sperm concentration.

Among the semen characteristics evaluated, the presence of gel in the ejaculate was 28%, a higher value than the 17% and 24.4% reported by Miranda [10] and Fallas et al. [38] but lower than the 32% reported by Mentado [9], studies conducted with rabbits of the same breed and under a similar reproductive rate. The presence of gel is considered a favorable sperm characteristic, as it contributes to greater ejaculate consistency and may be related to greater secretory activity of the accessory glands, as indicated by Onuoha [39]. The seminal volume found in supplemented rabbits 0.93 exceeds the averages reported by other authors 0.5, 0.8, 0.64, 0.66, 0.44 and 0.48 ml Castellini et al. [40], Rodriguez-De Lara [41], Niza et al. [36], Ferrian [42], Fallas [43], and Theu-Clément [44] respectively. Volume ranges between 0.3 and 0.9 ml are considered normal for rabbits [45]. The seminal pH recorded in this study was 7.0, a value within the range considered physiologically normal in rabbits (6.8–7.2), as Ferrián [42] reported. However, this value is slightly lower than that documented by other authors under different reproductive conditions. For example, Fallas-López et al. [38] reported a pH of 7.4 in animals managed under an extensive breeding rhythm, while Rodríguez-De Lara et al. [46] observed an even higher value, 7.6, in New Zealand White rabbits under a semi-intensive breeding rhythm.

The average sperm concentration per ejaculate was 285.5 million, a lower value than that reported by Fallas et al. [38], who documented 370 × 10^6 sperm in rabbits of the same breed under an extensive breeding rhythm. However, this figure is within the general range described for the species, which ranges from 90 to 477 million per ejaculate [47,48].

Yousef et al. [21] indicate that sperm quality varies with the collection order due to differences in epididymal sperm load, consistent with the 68.3% increase observed here.

Furthermore, the interaction between the male's physiological status, nutrition, and collection frequency can strongly modulate these parameters [11].

Several human studies have indicated that consumption of green tea with a high concentration of polyphenols increases lactate production in Sertoli cells, favoring the nutritional environment of germ cells in the seminiferous tubules and, consequently, reproductive health. These molecules have been shown to decrease the generation of reactive oxygen species (ROS) in reproductive tissues, which has been associated with improvements in sperm concentration, motility, morphology, and fertilization potential [49]. The present study's findings, which observed improved semen quality in males supplemented with green tea extract, could be related to these mechanisms. It supports the hypothesis that its antioxidant action directly improves rabbits' testicular microenvironment and spermatogenic functions.

Significant interaction effects between green tea extract treatment and ejaculation order were found on gel presence (P = 0.0001) and seminal volume (P = 0.013), as reported in Table 11. The highest gel presence was recorded in males supplemented with green tea during the first ejaculate (0.55 ± 0.08), representing an increase of 243.8% compared to the non-supplemented group and 450% compared to the second unsupplemented ejaculate. Regarding seminal volume, the highest value was also obtained in the green tea group in the first ejaculate (1.07 ± 0.04 ml), equivalent to an increase of 40.8% compared to the first ejaculate without green tea and more than double compared to the second unsupplemented ejaculate (0.42 ± 0.04 ml). Statistical analysis showed significant effects of the season (P = 0.007) and male weight at collection (P = 0.044) as covariates for seminal volume, adjusted to the following standard values: 1.68 and 4205.4, respectively.

Table 11. Least squares mean (± standard error) for interaction effects between green tea supplementation and ejaculate order on gel presence and seminal volume in New Zealand White rabbits.

Variable	Without Green Tea		With Green Tea		Valor-*P*
	First N = 43	Second N = 40	First N = 36	Second N =34	
Presence of ge	0.16 ± 0.06[b]	0.10 ± 0.06[c]	0.55 ± 0.06[a]	0.06 ± 0.06[c]	0.0001
Seminal volumen (mL) * °	0.76 ± 0.04b	0.42 ± 0.04c	1.07 ± 0.04a	0.50 ± 0.05c	0.013

abc – Letters with different superscripts in the same row differ (P<0.05).

A significant interaction (p = 0.013) was observed between green tea extract supplementation and ejaculate order on seminal volume. In both groups, the first ejaculate had larger volumes than the second, consistent with the expected physiology of reproductive males. However, males supplemented with green tea showed higher seminal volumes than the control group in both the first (1.07 mL vs. 0.76 mL) and the second ejaculate (0.50 mL vs. 0.42 mL). The greater slope in the line corresponding to the green tea treatment suggests its effect is more pronounced in the first ejaculate. This could be related to greater stimulation of initial accessory secretion induced by the supplement (Figure 8). On the other hand, Figure 9 shows a significant interaction (p = 0.0001) between green tea extract supplementation and ejaculation order on the presence of gel in semen. In both groups, the presence of gel was higher in the first ejaculate, which is consistent with the progressive depletion of accessory gland secretions in the second. However, in the first ejaculation, supplemented males presented a higher proportion of gel (0.55) compared to the control group (0.16), suggesting a stimulating effect of green tea on secretory activity. In contrast, in the second ejaculation, this advantage disappeared, even observing a lower presence of gel in the supplemented group (0.06) compared to the control (0.10), indicating that the supplement's effect is more evident in the initial phase of seminal emission. These findings highlight the importance of considering ejaculate order when evaluating the impact of nutraceutical supplements on semen quality.

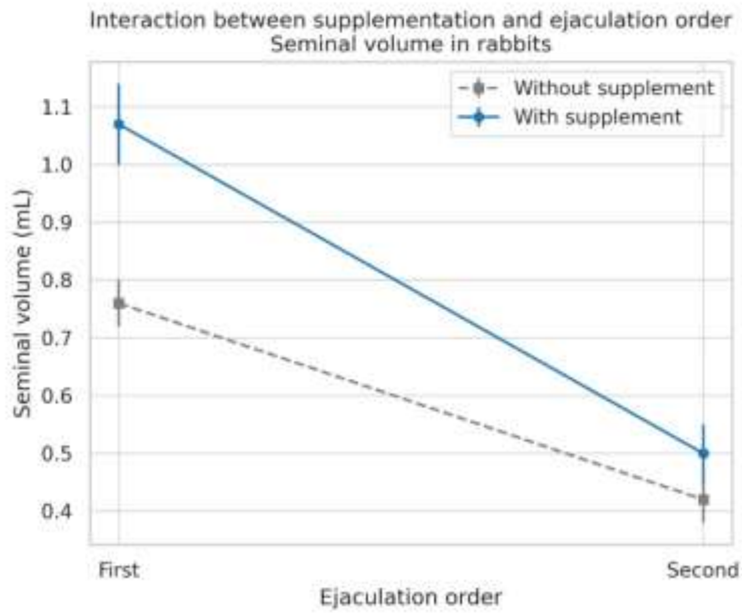

Figure 8.Interaction between supplementation and ejaculation order on seminal volume.

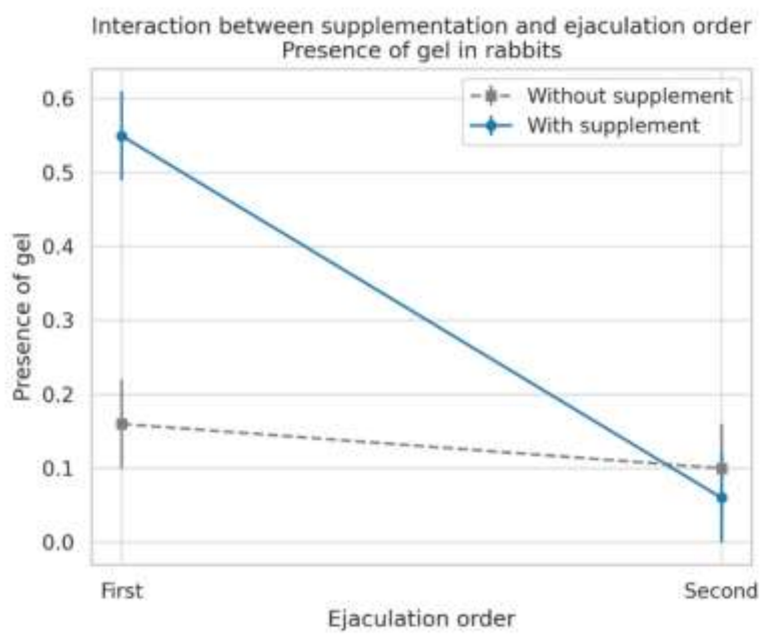

Figure 9..Interaction between supplementation and ejaculation order in the presence of gel.

These results reinforce the role of green tea extract supplementation as an effective strategy for improving key seminal parameters. The marked interaction observed suggests that the beneficial effect of green tea is enhanced when the male makes his first ejaculation after a rest period [9]. The greater presence of gel in the first ejaculation of rabbits fed green tea could be due to increased secretion of accessory glands stimulated by the antioxidant and anti-inflammatory effects of polyphenols, attributing these effects to an improvement in the secretory function of the reproductive system.

Furthermore, various studies agree that the first ejaculate is usually richer in sperm volume and quality due to the accumulation of epididymal and seminal secretions during rest [39]. This explains why, even without supplementation, the first ejaculate exceeded the second in gel presence and volume, which was even more pronounced with green tea.

Significant effects of green tea supplementation and ejaculation order were observed on various sperm motility and kinematic parameters, as detailed in Table 12. While no significant differences were found in sperm viability ($P = 0.292$), essential variations were detected in total motility ($P = 0.003$) and progressive motility ($P < 0.0001$), with higher values in the supplemented rabbits.

Supplementation also significantly improved VSL, VCL, STR, ALH, DSL, and DCL values. Ejaculation order significantly influenced VSL, VCL, LLN, STR, ALH, BCF, and DSL.

The covariate season was adjusted to a value of 1.68, as it was significant for the variables total motility ($p=0.003$), progressive motility ($p=0.022$), VSL ($P=0.019$), VAP ($p=0.0008$), STR ($p=0.0004$), WOB ($P=0.046$), ALH ($p=0.0001$), BCF ($p=0.007$), and DSL (0.018). Another variable that was adjusted was weight at the time of collection to a standard value of 4205.4 since it was significant for the variables VSL, VCL, VAP, WOB, ALH, DCL, DAP ($p=0.0001$), LIN ($p=0.025$) and DSL ($p=0.004$). Similarly, the covariate session was adjusted to a value of 11.38 as it was significant for the variables VCL, DCL, DAP ($P=0.0001$), VSL ($p=0.0006$), WOB ($p=0.002$) and DSL ($P=0.003$).

In the present study, total motility reached 89.3%, notably higher than the values reported in other works carried out under similar extensive reproduction conditions, where figures generally remain below 78%. For example, Arroita et al. [33] and Niza et al. [36] reported percentages of 70.9% and 70.5%, respectively, while Ferrián [42], Theau-Clément [44], and Rodríguez [41] reported even lower values, of 58.9%, 72%, and 77.4%. On the other hand, the results obtained are similar to those observed by Fallas [43], with 89.8%, and higher than the 82.8% recorded by Mentado [9]. Likewise, Miranda [10] reported a total motility of 87.4% in rabbits under the same experimental protocol, which supports the consistency and relevance of the findings of the present work.

The progressive motility recorded in this study was 46.2%, while Miranda [10] reported a slightly higher value of 53.9% under similar experimental conditions. This result also significantly exceeds the values observed by other authors, such as Fallas [43], Theau-Clément [44], and Rodríguez [41], who documented percentages of 37.5%, 42.6%, and 42.7%, respectively. The sperm viability observed in this study was 97.0%, a value similar to that reported by Miranda [10], who documented 94.1% under comparable conditions. This result stands out for being well above the 73.2% reported by Rodríguez [41], reflecting the high integrity and functionality of the sperm cells in the ejaculates evaluated.

Table 12. Least squares mean (± standard error) for the effect of green tea extract supplementation and ejaculate order on sperm viability, motility, and movement kinematics traits in New Zealand White rabbits.

	Treatment		Valor *P- Value*	Ejaculate Order		Valor - *P*
	Without green tea N = 83	Whit green tea N = 70		Firs N = 79	Secons N = 74	
Viability (%)	97.0 ± 0.25	97.5 ± 0.27	0.202	97.3 ± 0.25	97.2 ± 0.26	0.718
Total motility(%) *	88.9 ± 0.76	89.7 ± 0.83	0.446	88.9 ± 0.78	89.7 ± 0.81	0.482
Progressive motility(%) *	52.1 ± 1.61^{a}	40.3 ± 1.76^{b}	0.0001	48.7 ± 1.66^{a}	43.6 ± 1.71^{b}	0.034
VSL (µm/seg) ^ * °	76.1 ± 1.99	71.2 ± 2.17	0.102	74.8 ± 2.02	72.6 ± 2.09	0.455
VCL (µm/seg) ^ °	174.4 ± 2.85^{b}	189.0 ± 3.10^{a}	0.0008	178.6 ± 2.89	184.8 ± 2.99	0.142
VAP (µm/seg) * °	97.13 ± 2.18	102.0 ± 2.37	0.140	98.3 ± 2.22	100.8 ± 2.29	0.445
LIN (%) °	44.7 ± 0.91^{a}	39.6 ± 1.00^{b}	0.0003	43.4 ± 0.94	40.9 ± 0.97	0.069
STR (%) *	77.6 ± 0.97^{a}	70.2 ± 1.06^{b}	0.0001	75.5 ± 1.00^{a}	72.3 ± 1.03^{b}	0.030
WOB (%) ^ * °	55.5 ± 0.60	54.4 ± 0.66	0.203	55.4 ± 0.61	54.5 ± 0.63	0.307
ALH (mm) * °	7.06 ± 0.13	8.10 ± 0.14	0.0001	7.34 ± 0.13^{a}	6.93 ± 0.18^{b}	0.013
BCF (Hz) *	37.2 ± 0.36^{a}	35.1 ± 0.39^{b}	0.0001	36.3 ± 0.37	36.0 ± 0.38	0.549
DSL ^ * °	30.6 ± 0.99^{a}	27.6 ± 1.08^{b}	0.045	29.5 ± 1.00^{b}	28.7 ± 1.04^{a}	0.571
DCL ^ °	72.3 ± 1.57	76.5 ± 1.26	0.016	73.2 ± 1.18^{b}	75.6 ± 1.21^{a}	0.155
DAP ^ °	39.4 ± 0.90^{a}	40.5 ± 0.98^{b}	0.413	39.6 ± 0.92^{b}	40.3 ± 0.94^{a}	0.608

^ - A progressive collection session was used as a covariate.
* - Season was used as a covariate.
° - Weight at the time of semen collection used as a covariate.
ab - Letters with different superscripts in the same row differ (P < 0.05).

Regarding sperm kinematic characteristics, the straight-line velocity (SLV) averaged 73.65 µm/s in this study, slightly lower than that reported by Miranda [10], who documented 77.8 µm/s, but considerably higher than the values reported by Massányi et al. [50] and Safaa et al. [51], who recorded 35.8 µm/s and 31.5 µm/s, respectively. Similarly, the results obtained for the curvilinear velocity (CLV) were noticeably higher. In this study, 181.7 µm/s was reached, while Miranda [10] reported 170.3 µm/s. Both values greatly exceed the 99.9 µm/s and 74.1 µm/s described by Massányi et al. [50] and Safaa et al. [51], demonstrating a greater capacity for sperm movement under the conditions tested. Speeds >100 µm/s are more likely to reach and fertilize the oocyte [39]. The average trajectory velocity (ATV) recorded in this study was 99.57 µm/s, a value similar to that reported by Miranda [10], who obtained 98.1 µm/s, and close to the maximum range considered physiologically normal for the species. Although slightly lower than the value of 110.9 µm/s reported by Mentado [9], this result stands out for being well above the values documented by various authors, whose figures range between 42.5 and 60.8 µm/s Ferrian [42], Massányi et al. [50], Safaa et al. [51], and Rodríguez [41]. The literature indicates that the ATV in rabbits is usually between 36 and 108 µm/s, which places the results of the present study within the upper range reported for this variable [52]. The STR and LIN parameters, which describe the linearity and direction of movement, were significantly higher in the first ejaculation and the supplemented rabbits.

Higher values for these indices are associated with efficient trajectories and improved fertilization capacity [11].

Regarding the variables related to the direction of sperm movement, the linearity index (LIN) obtained was 42.15%, slightly lower than the 47.7% reported by Miranda [10] but comparable to the values of 50.4% and 49.2% reported by Safaa et al. [51] and Mentado [9] respectively. However, these results are far from the lower values recorded by Theau-Clément [44] and Rodríguez [41], who reported 40.8% and 42.5%. For the straightness index (STR), an average value of 73.9% was observed, while Miranda [10] reported 79.7%. This result exceeds the percentages of 71%, 69.9%, and 69.3% documented by Massányi et al. [50], Safaa et al. [51], and Rodríguez [41], respectively, and is in line with the highest levels described by Ferrián [42] and Mentado [9] with 81.3% and 80.1%. Finally, the recorded WOB index was 54.95%, compared to the 58.2% reported by Miranda [10]. This value is in line with the 59.5% documented by Mentado [9], is higher than the 51.0% described by Massányi et al. [50], but lower than the values of 66.9% and 67.8% reported by Lavara et al. [53] and Ferrián [42], respectively.

Table 13 presents the effects of treatment and ejaculation order on sperm morphology and reproductive potential. Although no significant differences ($p > 0.05$) were observed in the percentage of normal spermatozoa between treatments, rabbits supplemented with green tea showed a higher average value (+1.3%) than the control group. This same group presented lower percentages of abnormalities, such as sperm with bent tails and proximal droplets, which could reflect a beneficial effect of supplementation, although without statistical differences.

Regarding the number of normal motile progressive live sperm per ejaculate, the supplemented animals showed an average of 127.0×10^6 versus 94.1×10^6 in the control group, representing an increase of +32.9 million per ejaculate (+34.9%), although without reaching statistical significance ($p = 0.059$). Similar behavior was observed in the number of potential doses for AI, with an average of 25.4 doses in the green tea group versus 18.8 in the control group, equivalent to an absolute increase of 6.6 doses (+35.1%), also with a statistical trend ($p = 0.059$).

On the other hand, ejaculation order showed significant effects on the number of live, normal, progressive, motile sperm ($p = 0.059$), and especially on the number of AI doses ($p = 0.0001$). The first collection had an average of 29.6 doses, while the second was only 14.6, implying a 50.7% reduction, equivalent to 15 fewer doses in the second ejaculation.

Adjustments were applied for the covariate session (11.38), which was found to be significant for the variables normality ($p = 0.011$) and sperm with distal drop ($p = 0.28$). The covariate season was adjusted to a value of 1.68, which was significant for the variables normality (0.055) and normal, progressive, motile sperm per ejaculate ($p = 0.0001$). Likewise, the covariate weight at the time of collection was adjusted to a value of 4205.4 as it was significant for the variables normality ($p=0.004$), sperm with a distal drop ($p=0.0005$), and normal live progressive motile sperm per ejaculate ($p=0.049$). Green tea extract benefitted some morphological and reproductive aspects of the ejaculate, particularly in reducing sperm with abnormalities such as bent tails. This is consistent with the reports of El-Ratel et al. [1] and Hashem et al. [20], who highlight the antioxidant role of green tea in protecting sperm structures against oxidative damage. The increased number of potential doses per ejaculate in the supplemented group may have a direct impact on reproductive efficiency, who establish that a higher number of doses per ejaculate reduces costs and improves reproductive male utilization.

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Table 13. Least squares mean (± standard error) for the effect of treatment and ejaculate order on sperm morphology and reproductive potential in New Zealand White rabbits.

	Treatment			Ejaculate order		
	Without green tea N = 360	With green tea N = 316	*P-Value*	First N = 319	Second N = 357	*P-Value*
Sperm Morphology						
Normal Sperm(%) ^ * °	91.9 ± 0.82	92.2 ± 0.89	0.795	90.9 ± 0.83[b]	93.3 ± 0.86[a]	0.047
Sperm with Bent Tail (%)	3.22 ± 0.20[a]	2.48 ± 0.22[b]	0.015	3.00 ± 0.21	2.71 ± 0.22	0.332
Sperm with Curled Tail(%)	1.72 ± 0.14[a]	1.27 ± 0.15[b]	0.030	1.51 ± 0.14	1.47 ± 0.15	0.833
Sperm with Distal Drop(%) ^ °	8.40 ± 0.79	7.78 ± 0.86	0.601	9.47 ± 0.80[a]	6.72 ± 0.83[b]	0.019
Sperm with Proximal Drop(%)	0.49 ± 0.06[a]	0.63 ± 0.06[b]	0.096	0.57 ± 0.06	0.55 ± 0.06	0.766
Reproductive Potential						
Reproductive Potential ProgressiveSperm/Ejaculate($x10^6$) * °	94.1 ± 11.5[b]	127.0 ± 12.5[a]	0.059	148.0 ± 11.7[a]	73.1 ± 12.1[b]	0.059
Number of Doses for Artificial Insemination * °	18.8 ± 2.30[b]	25.4 ± 2.51[a]	0.059	29.6 ± 2.34[a]	14.6 ± 2.42[b]	0.0001

^ - Session order was used as a covariate.
° - Weight at the time of semen collection used as a covariate.
abc - Letters with different superscripts in the same row differ (P < 0.05).

4. Conclusions

Green tea extract supplementation in rabbits undergoing AI significantly improved key reproductive performance parameters, such as sexual receptivity, the number of live-born and weaned young rabbits, and litter weight at weaning. These results suggest that green tea may be a viable tool for optimizing reproductive efficiency in rabbit breeding. Although the effects in males were less consistent, a possible synergy was observed in specific treatment combinations. Further research into the optimal doses and physiological mechanisms is recommended to maximize its benefits without compromising the animals' reproductive health.

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4. CONCLUSIONES GENERALES

La suplementación con extracto de té verde puede ser una herramienta útil en programas reproductivos de conejos, al mejorar la respuesta sexual de las hembras, los índices de fertilidad, y parámetros clave de la calidad seminal en machos, contribuyendo así a la eficiencia global del sistema. Sus efectos parecen estar relacionados con sus propiedades antioxidantes, moduladoras del metabolismo y protectoras frente al estrés fisiológico. Sin embargo, para confirmar y comprender a profundidad los mecanismos involucrados, son necesarias investigaciones complementarias que incluyan estudios bioquímicos y moleculares que permitan cuantificar la actividad antioxidante a nivel celular. Esto incluye la evaluación de metabolitos específicos como especies reactivas de oxígeno (ROS), glutatión reducido (GSH), malondialdehído (MDA), enzimas antioxidantes y marcadores hormonales o de inflamación. Asimismo, es indispensable determinar los límites de seguridad en cuanto a la dosificación, estableciendo los umbrales a partir de los cuales el té verde deja de ser benéfico o incluso puede resultar perjudicial. También se recomienda estudiar sus posibles interacciones con otros suplementos naturales. En conjunto, el té verde se perfila como un aditivo funcional con potencial reproductivo, pero su implementación a gran escala requiere validaciones científicas más robustas, que integren pruebas de laboratorio, análisis a nivel celular y seguimiento a largo plazo en distintos sistemas productivos.

APÉNDICES

Apéndice 1.Configuración del sistema CASA.

Límites de análisis	Valor
Porcentaje mínimo de motilidad	0
Porcentaje mínimo de motilidad progresiva	0
Calibración	
Objetivo	Zeiss 10x CM-040GE:
Magnificación del objetivo X	1.2
Magnificación del objetivo Y	1.2
Cámara	
Exposición (Ms)	16
Integrado habilitado	Verdadero
Tiempo integrado	1000
Detección de células	
Elongación máxima (%)	100
Elongación mínima (%)	1
Mínimo brillo de la cabeza espermática	153
Tamaño máximo de la cabeza	91
Tamaño mínimo de la cabeza	4
Filtro estático de la cabeza	Falso
Brillo mínimo de la cola	255
Desplazamiento automático del brillo mínimo de la cola	10
Modo de brillo mínimo de la cola	Manual
Cámara espermática	
Correcciones capilares	1.3
Profundidad de la cámara (µm)	20
Tipo de cámara	Capilaridad
Iluminación	
Tipo de iluminación	Fluorescencia
Intensidad de la fluorescencia	3996
Intensidad visible	2953
Fotómetro máximo	90
Fotómetro mínimo	70

Apéndice 2.Configuración sistema CASA para cinemática de movimiento espermático.

Cinemática	Ajuste
Índice de rectitud (STR) progresiva (%)	80
Velocidad promedio de trayectoria (VAP) progresiva (µm/s)	50
VAP lento (µm/s)	20
Velocidad rectilínea (VSL) lenta (µm/s)	30
Algoritmo estático	Largo
VAP estático (µm/s)	4
VSL estático (µm/s)	1
Multiplicador de ancho estático	0.5
Morfología	
Intervalo de confianza del Reflejo de la pieza media distal (DMR) (%)	25
DMR Máximo de la gota a la cola (µm)	5
DMR Longitud máxima de la cola (µm)	20
Intervalo de confianza de las gotas (%)	20
Distancia mínima de gota distal (µm)	4
Largo de la cabeza gota proximal (µm)	10.5
Largo mínimo de la cola (µm)	0
Largo promedio angular de la cola doblada	5
Tasa mínima de doblamiento angular	5
Intervalo de confianza de las colas dobladas (%)	50
Angulo mínimo de la cola enrollada (°)	180
Intervalo de confianza de la cola enrollada (%)	50
Intervalo de confianza de la cola (%)	75
Estado	
Estado de temperatura	37
Categoría Viadent	
Esperma viable fluorescencia	Viable
Captura de video	
Velocidad del marco de captura (Hz)	1
Conteo del marco	30