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EFFECT OF CONJUGATED LINOLEIC ACID ON EMBRYO QUALITY IN NEW ZEALAND RABBITS

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Abstract: One of the main challenges in commercial rabbit production is the high nutritional demands in primiparous females, which, together with their limited intake capacity, reduces their body fat stores and could be partly responsible for their low reproductive performance. Supplementation strategies with rich sources of polyunsaturated fatty acids in primiparous rabbits could compensate for their low reproductive performance. Therefore, the aim of the present study was to evaluate the addition of increasing levels of conjugated linoleic acid (CLA) to the diet of rabbits on embryo development and embryo quality in New Zealand rabbits. Thirty New Zealand females aged 28-30 d and weighing an average of 587.5±35 g at the start of the experiment were randomly distributed into three feeding treatments: T0: (n=10) commercial diet, TCLA1 (n=10): commercial feed +2.5% CLA, TCLA2 (n=10): commercial feed +5.0% CLA, and were fed *ad libitum* with commercial balanced feed containing 18% crude protein and 12% crude fibre during the experimental phase (120 d). The females were exposed to a male for mating and sacrificed at 72 h post-coitus to obtain the embryos. No significant differences (P>0.05) were observed in the final weight of the animals between treatments. However, females fed with CLA presented a higher body condition compared to the control group (P<0.05). The TCLA2 group females presented an average of 9.5±1.467 embryos recovered per female, showing a significant difference (P<0.05) compared to the TCLA1 and T0 treatments. The embryos from the TCLA1 and TCLA2 treatments presented a higher percentage of excellent and good quality embryos compared to the control group (P<0.05). In conclusion, supplementation with 2.5 and 5.0% conjugated linoleic acid does not affect the final weight of female rabbits but significantly improves their body condition, the number of embryos recovered and embryo quality. These results highlight the potential of CLA as a nutritional tool to optimise reproductive efficiency in rabbit production systems.

Key Words: conjugated linoleic acid, embryo quality, productive performance.

INTRODUCTION

The term conjugated linoleic acid (CLA) refers to a group of isomers of linoleic acid (Lapa *et al.*, 2011). Researchers have discovered about 28 isomers of CLA. The biological functions of the cis 9, trans-11, trans-10 and cis 12 isomers are the most important (Kennedy *et al.*, 2009), as they can control fat metabolism, lower cholesterol, stop atherosclerosis, boost immunity, raise bone density, stop diabetes and help animals grow (Chen *et al.*, 2019). Vegetable oils contain small amounts of CLA, but products like meat and milk have a particularly high concentration of it, up to 0.60-0.80% of total lipids (Yurawecz *et al.*, 1998), depending on ante- and postmortem factors such as

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breed, climate, intestinal health, type of production, muscle type and raw materials. Research has demonstrated the positive impact of polyunsaturated fatty acids (PUFA) on various aspects of reproductive function (Kujoana *et al.*, 2024). In the specific case of rabbits, researchers describe effects on lipid metabolism, steroid hormone secretion in females and a favourable impact on reproductive tissues and gametogenesis, all of which are prerequisites for excellent fertility and survival rates (Rodríguez *et al.*, 2019). Nulliparous rabbits are particularly vulnerable to this negative energy imbalance, as their growth is not yet fully completed. This energy deficit significantly impacts their body condition and hormonal status, resulting in reduced fertility and, ultimately, a shorter productive life. (Xiccato *et al.*, 2004, Menchetti *et al.*, 2020, Quattrone *et al.*, 2024). Therefore, considering supplementation strategies with rich sources of PUFA in primiparous rabbits could compensate for their usually observed poor reproductive performance. According to Rebollar *et al.* (2014), giving animals n-3 PUFA in their food for a long time does not change how much they eat and seems to improve the endocrine function of the corpora lutea during the implantation period (days 5 to 7 after artificial insemination), which leads to bigger and heavier litters and lower mortality rates at the second parturition. Consuming these acids during fertilization and early pregnancy also changes the oviductal environment, which impacts the early stages of embryonic development. A steady and high level of PUFA (n-3) during these times may have unknown effects on fertilization and early embryonic development (Fabjanowska *et al.*, 2023). *In vitro* studies show that supplementation of culture media with the trans-10, cis-12 isomer reduces excessive lipid accumulation, decreasing the hyperlipidaemia that characterises *in vitro* cultured embryos, improving their resistance and tolerance to manipulation, increasing the competence of bovine oocytes to develop into better quality embryos (Abazarikia *et al.*, 2020), and allowing them to develop a selective process to guarantee their protection and avoid the risk of cell damage (Zeng *et al.*, 2023). Therefore, the present study aimed to assess the impact of increasing levels of conjugated linoleic acid in the rabbit's diet on embryo development and quality in New Zealand rabbits.

MATERIALS AND METHODS

Location

The present study was conducted in the Reproductive Biotechnology laboratory of the University of Papaloapan. Located in the Papaloapan Basin Region, at the coordinates Latitude North 18°04'52" and Longitude West 96°07'07", at an altitude of 20 metres above sea level (INEGI, 2015).

Animals and diets

Thirty female New Zealand rabbits, aged 28-30 d, with an average weight of 587.5±35 g at the onset of the experiment, were randomly distributed into three feeding treatments: T0: (n=10) commercial diet, TCLA1 (n=10): commercial food +2.5 % CLA equivalent to 2.5 g per 100 g of food; TCLA2 (n=10): commercial food +5.0% CLA equivalent to 5 g per 100 g of food consumed. A commercial dietary supplement containing conjugated linoleic acid (CLA) was used in this study (GNC Total Lean®, GNC Holdings Inc., Pittsburgh, PA, USA).

Females were housed in individual cages and fed *ad libitum* on commercial feed containing 18% crude protein and 12% crude fibre during the experimental phase (120 d). To ensure that each female consumed the correct amount of CLA, it was administered orally with the aid of a syringe on an individual basis. The animals were weighed at the beginning and end of the experiment after a 12 h fasting period with a Torrey PCR20 digital scale.

Body condition

The body condition score (BCS) of the rabbits was assessed by manual palpation, according to the criteria described by Reusch (2010). This procedure focused on palpation of the ribs in the thoracic region posterior to the elbow, as this area sensitively reflects changes in subcutaneous fat deposits, whereas visual inspection may be unreliable due to variations in coat type and length. Body condition scoring was assigned on a five-level scale:

Score 1 (Worn): Pelvis and ribs very easily palpable with extremely sharp edges, tactile sensation resembling a "bag full of rulers"; concave rump.

Score 2 (Thin): Pelvis and ribs easily palpable and sharp, with flat rump.

Score 3 (Ideal): Palpable pelvis and ribs with rounded edges, "pen bag" feel and flat croup.

Score 4 (Fat): Firm pressure needed to palpate ribs, with rounded rump.

Score 5 (Obese): The ribs are not palpable and the rump has an accentuated convexity.

The evaluation was performed by a single evaluator, which ensured consistency and reduced interobserver variability.

Mating and embryo collection

The females were exposed to a male for mating and sacrificed 72 h post-coitus by intravenous administration of 50 mg of sodium thiopental. The experimental protocol was approved (UNPA/CBE/025) by the Bioethics Committee of the University of Papaloapan. The animals were treated humanely and with respect for suffering. The reproductive tracts were placed in a saline solution with an isotonic concentration of sodium chloride (NaCl) at 0.9% at a temperature of 37°C; the oviduct and the first third of the uterine horn were rinsed with 2.5 mL of Dulbecco's phosphate-buffered saline. (DPBS, Sigma-Aldrich).

Embryo classification

Morphological evaluation of the embryos was performed by a single experienced evaluator, who carried out the observations without knowledge of the treatment groups to ensure objectivity and avoid interobserver variability. Each recovered embryo was examined individually with an inverted microscope (Nikon Eclipse TS100) coupled to a high-resolution digital camera. Q-Capture Pro software (QImaging, Surrey, Canada) was used to capture and analyse the images, and scales calibrated with reference micrometres were used to ensure accuracy of diameter measurements. Embryo grading was performed based on criteria established by Linder and Wright (1983), dividing embryos into four groups: Grade 1 (Excellent) for round, uniform embryos with consistent cells; Grade 2 (Good) for embryos with minor shape problems and some bubbles; Grade 3 (Fair or Poor) for embryos with notable problems and damaged cells; and Grade 4 (Non-transferable) for embryos with severe structural problems, many bubbles and significant cell damage. The evaluations were performed on all the embryos recovered.

Statistical analysis

The initial live weight, final live weight and average daily gain (ADG) variables were analysed by analysis of variance (ANOVA) according to the following model: $Y_{ijk} = \mu + t_i + \varepsilon_{ijk}$, where: Y_{ijk}: response variables; μ: population mean; t_i: treatment effect; ε_{ijk}: experimental error. Embryo quality and body condition data were subjected to a chi-square test using SPSS Statistic Version 2.3 at a significance level of ($P<0.05$).

RESULTS AND DISCUSSION

Growth parameters of rabbits fed CLA are shown in Table 1. No significant differences ($P>0.05$) were observed in body weight at the start of the experiment between treatments (30 d of age). At the end of the experimental period, the final weight of the animals between treatments showed no difference ($P>0.05$), with the overall mean being 2.75±0.216 kg and daily weight gain of 35.1±3.3 g. However, females fed CLA showed a higher body condition compared to the control group ($P<0.05$).

Table 1: Productive performance of New Zealand rabbits supplemented with conjugated linoleic acid (CLA).

	Treatments				
Variables	T0	TCLA1	TCLA2	EEM	P
IBW (g)	590.3	585.7	536.5	115.0	0.523
FBW (g)	2725	2865	2678	216	0.634
ADG (g/d)	32.8	36.7	35.9	3.3	0.546
BCS	2.6[a]	3.1[b]	3.4[b]	0.4	0.045

[ab] Means with different superscripts within the row are different ($P<0.05$).

Treatments: T0: control group; TCLA1: animals supplemented with commercial food +2.5% CLA; TCLA2: animals supplemented with commercial food +5.0% CLA

Variables: IBW: Initial body weight; FBW: Final body weight; ADG: Average daily gain; BCS: Body condition score.

Table 2: Cell number and embryo diameter at 48 h post-coitus recovered from conjugated linoleic acid (CLA) supplemented New Zealand rabbits.

Treatments	Number of cells	Diameter (µm)
T0	16.30 ± 0.514[a]	128.7±1.513[a]
TCLA1	16.80 ± 0.586[a]	129.2±0.927[a]
TCLA2	20.30 ± 0.447[b]	138.1±1.996[b]

[ab] Means with different superscripts within the column are different (P<0.05).

Treatments: T0: control group; TCLA1: animals supplemented with commercial food +2.5% CLA; TCLA2: animals supplemented with commercial food +5.0% CLA.

Figures 1 and 2 show the individual number of embryos recovered per female and the total number of embryos obtained per treatment, respectively. The group of females that consumed 5.0% CLA presented a higher number of embryos recovered; the average number of embryos recovered was 9.5±1.35, showing a significant difference (P<0.05) compared to the TCLA1 and T0 treatments (7.8±1.49, 7.6±1.07, respectively). Likewise, embryos recovered from the TCLA2 treatment showed a higher number of cells and a larger diameter of 10 µm compared to the T0 and TCLA1 treatments (P<0.05) (Table 2). Embryo quality is shown in Figures 3 and 4. Embryos from females that consumed 2.5 and 5.0% CLA had a higher percentage of excellent (38.5 and 40%) and good quality (31.6 and 28.2%) embryos, respectively, compared to the control group (26.3%) (P<0.05). The control group presented a higher percentage of embryos of regular and poor quality (41.1%) compared to the TCLA1 (33.3%) and TCLA2 (26.7%) groups, finding a significant difference between treatments (P<0.05)

DISCUSSION

CLA is a high-energy fatty acid, recognised for its role in influencing animal growth performance (Wu *et al.*, 2016; Wang *et al.*, 2020). The impact of dietary supplementation with CLA varies depending on the dose and age of the animal (Corino *et al.*, 2004). Its effects have been reported on body composition in pigs (Pinelli-Saavedra *et al.*, 2019), broilers (Cardinal *et al.*, 2020), rabbits (Jamal and Zaza, 2015; Abdelatty *et al.*, 2019) and rats (Martín-González *et al.*, 2020).However, the degree of response appears to be species-specific and dose-dependent (Whigham *et al.*, 2007).

In this study, dietary supplementation with 2.5 and 5.0% CLA did not modify final live weight or average daily gain in New Zealand rabbits, which is consistent with those reported by other authors (Marounek *et al.*, 2007; Abdelatty *et al.*, 2019; Liu *et al.*, 2022). This observation supports the notion that CLA supplementation can modify body composition by reducing adipose tissue without negatively affecting growth rates (Li *et al.*, 2005). Some studies

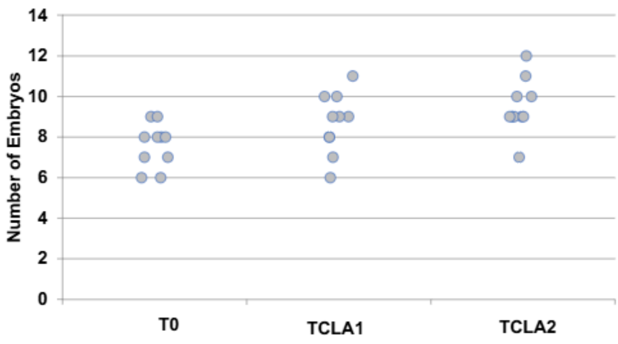

Figure 1: Individual number of embryos recovered from New Zealand rabbits. Treatments: T0: control group; TCLA1: animals supplemented with commercial food +2.5% CLA; TCLA2: animals supplemented with commercial food +5.0% CLA: conjugated linoleic acid.

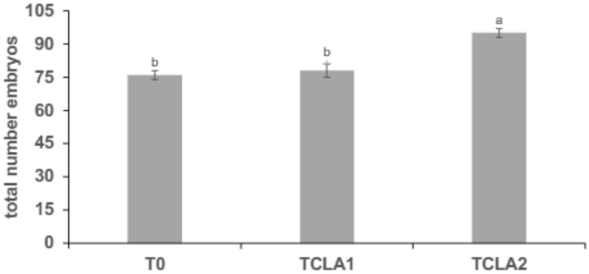

Figure 2: Total number of embryos recovered in New Zealand rabbits supplemented with conjugated linoleic acid (CLA). [ab] Means with different superscripts between treatments are different (P<0.05). Treatments: T0: control group; TCLA1: animals supplemented with commercial food +2.5% CLA; TCLA2: animals supplemented with commercial food +5.0% CLA.

in rabbits (Sirri *et al.*, 2010) suggest that CLA may slightly limit weight gain, probably due to alterations in energy partitioning and reduced efficiency in the conversion of dietary energy into adipose tissue. These effects could be partially associated with the impact of CLA on gut fermentation and microbiota composition (Marounek *et al.*, 2002).

Importantly, females supplemented with CLA showed a significant improvement in body condition compared to controls. Body condition, defined as the amount of metabolizable energy stored in adipose and muscle tissues (Nazhat *et al.*, 2021), is a crucial indicator of reproductive potential. Studies in other species have shown that CLA can modulate lipid metabolism and fat deposition, improving body composition without compromising muscle mass (Ramiah *et al.*, 2014; Fernández-Fígares *et al.*, 2019; Rao *et al.*, 2023; Panisson *et al.*, 2020). These results reinforce the idea that improving body condition through dietary interventions could positively influence reproductive parameters in rabbits.

In terms of reproductive performance, rabbits supplemented with CLA, particularly at 5.0%, showed significantly higher numbers of recovered embryos. This result is consistent with the proposal that dietary lipids act as modulators of the reproductive environment, promoting follicular development, ovulation and early embryonic viability

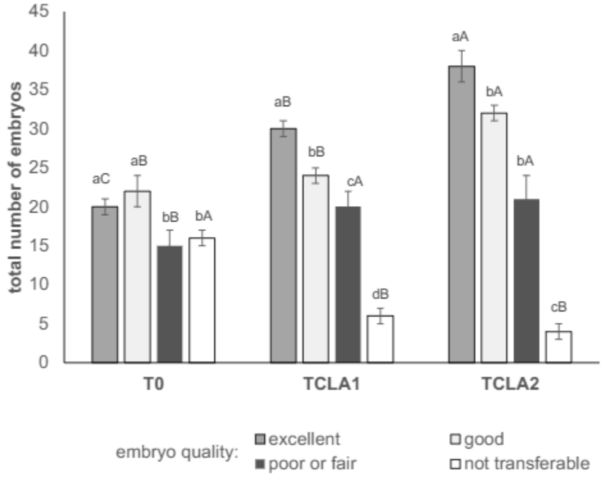

Figure 3: Embryo quality of New Zealand rabbits supplemented with CLA. [A,B,C] Means with different superscripts between treatments were different at P<0.05. [abc] Means with different superscripts within treatments were different at P<0.05. Treatments: T0: control group; TCLA1: animals supplemented with commercial food +2.5% CLA; TCLA2: animals supplemented with commercial food +5.0% CLA: conjugated linoleic acid.

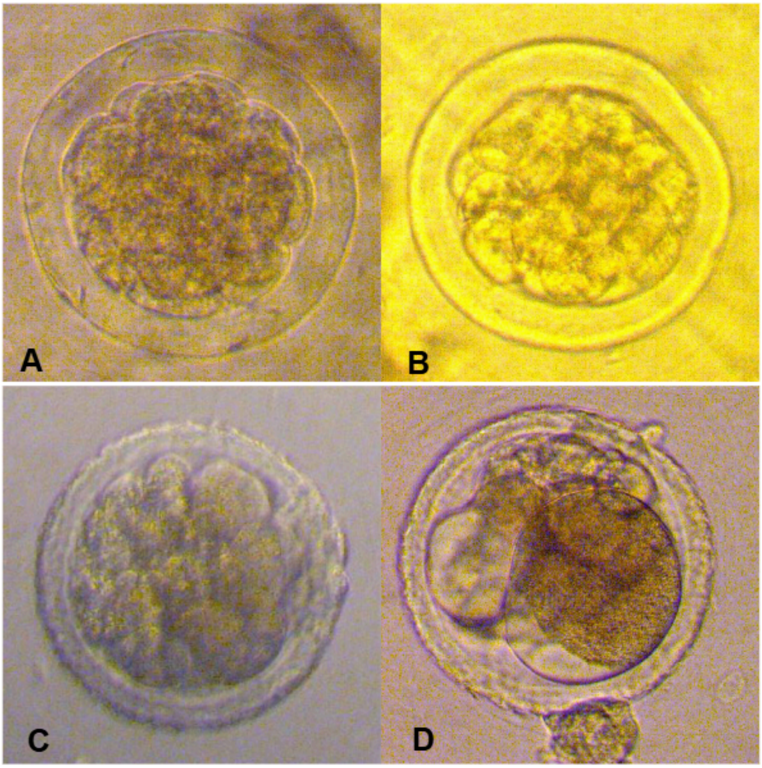

Figure 4: Evaluation of embryo quality in New Zealand rabbits according to morphology. Representative images of rabbit embryos morphologically evaluated according to their quality: A) Embryos of excellent quality, with symmetrical blastomeres; B) Embryos of good quality, with slight structural irregularity; C) Embryos of regular quality, with heterogeneous blastomeres and initial signs of degeneration; D) Embryos of poor quality, loss of shape and poorly defined cellular structures.

(Lucy *et al.*, 1992). Although most of the evidence comes from ruminants (Zachut *et al.*, 2010; Moussavi *et al.*, 2007; Elis *et al.*, 2016; Mahla *et al.*, 2017, 2023), the present study provides evidence suggesting similar benefits of lipid supplementation on reproductive efficiency in rabbits. The increase in embryo number could be related to improved energy reserves and metabolic signalling pathways involved in ovarian function, although the exact mechanisms in rabbit require further elucidation.

In addition, embryos derived from CLA-supplemented females showed a higher proportion of excellent and good quality grades compared to controls. Fatty acids such as oleic acid, palmitic acid and linoleic acid have been shown to promote rabbit embryo development from the single cell stage to morula (Kane, 1979). The improvement in embryo quality observed in this study could reflect improved cytoplasmic maturation of the oocyte and more efficient early segmentation events, potentially influenced by favourable changes in the lipid composition of the follicular environment (Khandoker and Tsujii, 1999).

Previous studies have shown that CLA can improve embryo quality *in vitro* by reducing lipid accumulation in the cytoplasm, increasing membrane fluidity and improving resistance to cryopreservation (Carvalho *et al.*, 2019; Accorsi *et al.*, 2015; Leão *et al.*, 2015). Although the present study was performed *in vivo*, it is plausible that similar mechanisms contributed to the observed improvements in embryo quality. In addition, the antioxidant properties of CLA (Abazarikia *et al.*, 2020; 2021; Zhang *et al.*, 2024) could have reduced oxidative stress during oocyte maturation and early embryo development, favouring better developmental competence.

Taken together, the findings of this study demonstrate that CLA supplementation in the diet of young rabbits improves body condition, increases the number of embryos recovered and improves embryo quality. These results support the use of CLA as a potential nutritional strategy to optimise reproductive performance in rabbit production systems. However, more research is needed to determine optimal doses, duration of supplementation and long-term effects on metabolic health and reproductive sustainability.

CONCLUSIONS

Supplementation with 2.5 and 5.0% conjugated linoleic acid does not affect the final weight of female rabbits, but significantly improves their body condition score, number of embryos recovered and embryo quality. These findings highlight the potential of CLA as a nutritional tool to optimise reproductive efficiency in rabbit production systems.

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Conflict of interest: The authors declare that there is no conflict of interest.

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