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Research Paper

The Effects of N-acetyl Tryptophan and Omega-3 Feeding During the Transition Period on Relative Expression of Genes Related to Endocannabinoid System in Adipose Tissue of Holstein Cows

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ABSTRACT

Introduction: The transition period in dairy cows is marked by significant metabolic, endocrine, and immunological transformations, often contributing to negative energy balance and enhanced susceptibility to metabolic diseases. The endocannabinoid system (ECS) contributes to regulating energy homeostasis and inflammation, and its activation during this period has been implicated in insulin resistance and reproductive dysfunction.

Materials & Methods: This research investigated the impact of dietary supplementation with omega-3 fatty acids (100 g/cow/day) and tryptophan (TRP) amino acid (100 g/cow/day), and the interaction of these components, on ECS-related gene expression in Holstein dairy cow adipose tissue (AT) at day 21 and 42 postpartum. Cows underwent presynchronization with two injections of prostaglandin F2α (PGF2α) administered 14 days apart. Following the second PGF2α injection, an Ovsynch protocol was started with a GnRH injection; all cows were artificially inseminated, and pregnancy was assessed via ultrasonography on day 33 post-insemination. A designated area of 25 cm² (5×5 cm) was shaved and disinfected, and a scalpel was used to collect the subcutaneous AT sample. The obtained sample was rinsed with distilled water, and then placed into a microtube and stored at -80 ˚C for measuring the relative expression of genes related to the ECS: cannabinoid receptor 1 (CNR1), cannabinoid receptor 2 (CNR2), fatty acid amide hydrolase (FAAH), monoglyceride lipase (MGLL), N-acyl phosphatidylethanolamine phospholipase D (NAPEPLD), N-acylethanolamine acid amidase (NAAA), cyclooxygenase 2 (COX2).

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Results: The findings showed that the interaction of omega-3 and TRP supplementation upregulated the relative expression of *ECS*-related genes, such as NAAA, FAAH, and MGLL, and downregulated the expression of *CNR2* and *NAPEPLD*, with no significant effects on *CNR1* and *COX2* on day 21 and 42 postpartum.

Conclusion: These results demonstrate a synergistic effect between omega-3 and TRP in modulating ECS activity, suppressing inflammation, and enhancing lipid metabolism. The downregulation of lipogenic and pro-inflammatory genes observed in these results supports that targeted nutritional changes can modulate ECS activity, improve negative energy balance, and enhance metabolic conditions in transition dairy cows.

1. Introduction

The transition period in dairy cows, spanning three weeks before to three weeks after parturition, represents a pivotal phase for both health and productivity [1]. This period is marked by profound alterations in metabolic, endocrine, and immune functions. Inadequate nutrient intake during the prepartum phase, combined with heightened nutrient demands for fetal development, mammary tissue expansion in late gestation, and the initiation of colostrum and milk synthesis postpartum, typically culminates in a state of negative energy balance. To counteract this, the mobilization of endogenous reserves—including lipids, proteins, and glycogen—becomes indispensable. The extent of body weight loss during this period appears to be an inherent trait, potentially underpinned by genetic factors, particularly in high-yielding dairy cows [1]. Consequently, cows with the highest milk output at the onset of lactation often experience pronounced negative energy balance, triggering the breakdown of body reserves, notably from adipose tissue (AT).

The endocannabinoid system (ECS) plays a significant role in modulating energy metabolism, immune responses, and reproductive functions in mammals through endocannabinoid ligands, metabolic enzymes, and cannabinoid receptors [2]. In mammals, the cannabinoid-1 receptor (CNR1) is widely distributed in both the central nervous system (CNS) and peripheral tissues, where its activation promotes anabolic processes. Conversely, the cannabinoid-2 receptor (CNR2) is predominantly expressed in immune cells and is primarily associated with anti-inflammatory effects upon activation. The activation of CNR1 receptors by eCBs promotes insulin resistance by impairing central insulin sensitivity and decreasing insulin's anorexic effects. Furthermore, excessive CNR1 signaling disrupts metabolic homeostasis by reducing secretion of gastric inhibitory polypeptide (GIP), enhancing pancreatic inflammation and β-cell dysfunction, impairing hepatic insulin clearance, and exacerbating systemic insulin resistance [2]. Such disturbances can exacerbate the state of negative energy balance, prolonging fat mobilization and increasing the risk of hepatic lipidosis and systemic inflammation [3]. These outcomes not only hinder the restoration of energy homeostasis but also compromise the animal's overall metabolic resilience during this physiologically demanding period. In addition to its metabolic effects, excessive ECS activity has been implicated in reproductive dysfunction. Elevated endocannabinoid levels can negatively affect the hypothalamic–pituitary–gonadal axis, disrupt luteinizing hormone (LH) secretion, and impair follicular development and ovulation [4]. In dairy cows experiencing negative energy balance, this may contribute to delayed resumption of ovarian cyclicity and reduced fertility postpartum. Moreover, ECS-mediated inflammatory responses in reproductive tissues can further impair uterine health and embryo implantation [5]. Together, these findings suggest that heightened ECS activity during the transition period may not only worsen metabolic imbalance but also negatively impact reproductive efficiency, highlighting the need for strategies to modulate this system in a controlled manner.

Tryptophan (TRP) is classified as an essential amino acid, that significantly affects feed consumption, growth efficiency, reproductive health, neural activity, immune response, and stress management in both monogastric and ruminant species [6]. Additionally, free plasma TRP has several functions, acting as a protein component and a precursor for various metabolites, including the neurotransmitters serotonin, melatonin, and niacin [7]. TRP metabolites are involved in the modulation of inflammation and insulin resistance [8]. TRP also exerts notable immunomodulatory effects. Through its interaction with the ECS—particularly by influencing the CNR2 receptor expression on immune cells—TRP may enhance anti-inflammatory signaling. The reduction in inflammatory markers seen in the TRP-supplemented group supports this proposed mechanism [8].

Omega-3 fatty acids, also known as n-3 fatty acids, comprise a category of polyunsaturated fatty acids, that are well recognized for their anti-inflammatory effects, largely through the production of specialized pro-resolving lipid mediators and modulation of nuclear receptors [10]. Research conducted by Akhtar et al. (2024) suggests that supplementing with omega-3 PUFAs may improve fertility by boosting conception rates, supporting healthy follicle development and ovulation, and enhancing key reproductive functions like corpus luteum formation and steroidogenesis [11]. Omega-3 fatty acids also play a key role in moderating lipid metabolism during the transition period, particularly by reducing excessive fat mobilization from AT. During early lactation, cows experience negative energy balance, leading to elevated non-esterified fatty acids (NEFAs) in the blood, which increases the risk of hepatic lipidosis and ketosis. Omega-3s may modulate the ECS, which is implicated in the regulation of appetite and lipid metabolism, thereby reducing the over-activation of lipolysis during negative energy balance [12]. This regulatory effect supports a more balanced energy metabolism, lowers the accumulation of NEFAs and triglycerides in the liver, and contributes to improved metabolic health and productivity in early lactation.

Moreover, omega-3 fatty acids demonstrate immunomodulatory effects through nutrigenomic mechanisms, downregulating pro-inflammatory pathways while enhancing immune regulation [13]. Additionally, omega-3s influence reproductive performance by altering prostaglandin synthesis, reducing luteolysis, and potentially enhancing embryo survival [14].

Numerous research studies have indicated that dietary omega-3 fatty acids have beneficial impacts on the reproductive and physiological functions of dairy cows [14-17]. Modifying the dietary ratio of omega-6 (n-6) to n-3 is a recognized approach to influence the ECS [19]. In dairy cows, AT and the liver are crucial for energy metabolism, and the ECS may play a significant role in regulating lipogenesis and adipogenesis, as well as inhibiting lipolytic processes [18]. Previous findings confirmed the presence of essential ECS components in the subcutaneous AT of dairy cows, with increased levels of endocannabinoids detected in AT during the postpartum phase [20]. Additionally, the ECS may be linked to inflammation in the AT of dairy cows, as numerous inflammatory mediators have been identified in cows experiencing high lipolysis postpartum, along with heightened expression of cannabinoid receptor-2 (CNR2) and the enzymes necessary for the synthesis and breakdown of anandamide (AEA) [12, 17].

The current study aims to explore the effects of N-acetyltryptophan and omega-3 feeding during the transition period on the relative expression genes related to the ECS in AT of Holstein cows.

2. Materials and Methods

2.1. Experimental treatments

This research took place at the Dashte-Naz Dairy Farm in Sari, Mazandaran Province. A total of 120 multiparous pregnant Holstein cows were selected based on their expected calving dates and randomly assigned to one of four treatment groups. The cows participated in the study from 30 days prior to calving until 80 days post-calving, utilizing a 2×2 factorial design with two independent variables: TRP (0, 100) and omega-3 (0, 100). In order to investigate endocannabinoid gene expression, AT samples were collected from 16 cows (four replicates per treatment).

2.2. Estrous synchronization

Cows underwent presynchronization with two injections of prostaglandin F2α (PGF2α) administered 14 days apart, beginning on day 30 postpartum. Following the second PGF2α injection, an Ovsynch protocol was started with a GnRH injection. Sixteen hours after the second GnRH injection, all cows were artificially inseminated. On day 26 post-insemination, a GnRH injection was given to all cows, and pregnancy was assessed via ultrasonography on day 33 post-insemination. Non-pregnant cows received PGF2α on the day of pregnancy diagnosis, followed by a GnRH injection two days later, and were inseminated 16 hours after the final GnRH injection [22]. Cows confirmed pregnant after the first or second insemination on day 33 had their pregnancy verified.

2.3. Subcutaneous AT sampling

Subcutaneous AT was collected from the pin region following the procedure outlined by Zachut et al. [17]. A designated area of 25 cm² (5×5 cm) was shaved and disinfected with iodine-based antiseptics. A scalpel was used to create a 2.5 cm incision, allowing for the aseptic collection of the subcutaneous fat sample with surgical forceps. The obtained sample was rinsed with distilled water, dried, and placed into a microtube. This microtube was then promptly immersed in liquid nitrogen and transported to the laboratory, where it was stored at -80 ˚C for subsequent relative gene expression analysis. To mitigate the risk of infection at the sampling site, an immediate administration of 650 mg of ceftiofur 5% antibiotic (Ceftiofur 5%, Cosima International Industrial Company, Chicago, USA) was performed post-sampling.

2.4. Real-time polymerase chain reaction (PCR)

2.4.1. RNA isolation from AT

Total RNA was extracted from ATs utilizing the Parstous Total RNA Extraction Kit (A101231). This kit comprises two solutions (PW and RL), RNase-free water, a collection tube, and a spin column. After homogenizing AT, it was lysed in RL buffer, mixed with chloroform, and centrifuged to separate phases. 400 μL of the aqueous phase was transferred to a 1.5 mL tube, an equal volume of cold 70% ethanol was added, and the mixture was loaded onto spin columns and centrifuged at 13,000×g for 1 minute. RNA was column-purified using 700 μL PW wash buffer, followed by centrifugation at 13,000×g for 1 minute. 50 μL of RNase-free water was added to the column membrane and RNA was eluted by centrifugation at 13,000×g for 1 minute. The total RNA isolated from the samples was stored at -70 ˚C until cDNA synthesis.

2.4.2. DNase treatment of extracted RNA samples

To remove double-stranded DNA contamination and purify the RNA for high-quality results, the extracted RNA samples underwent treatment with the commercial enzyme deoxyribonuclease (DNase) utilizing the DNase I Kit (YTA, YT9058).

2.4.3. cDNA synthesis method

The synthesis of cDNA was carried out using the Easy cDNA Supra-TM Synthesis Kit. A 10 μL aliquot of Buffer-Mix and 1 μL of Supra Enzyme Mix were added to a 0.2 mL tube containing RNA. The reaction was incubated at 25 ˚C for 10 minutes, followed by 50 ˚C for 30 minutes, and the reaction was completed by heating to 85 ˚C for 5 minutes to inactivate the enzyme. The resulting cDNA was preserved at -70 ˚C until it was needed for relative gene expression analysis through real-time PCR.

2.4.4. Real-time PCR reaction

The real-time PCR reaction was conducted with specific primers on the Roche LightCycler 96 instrument. The real-time PCR mixture consisted of cDNA, SYBR Green Master-mix, with a total volume of 15 μL, including 1 μL cDNA, 7.5 μL SYBR Green Master-mix, 0.7 μL forward primer, 0.7 μL reverse primer, reverse primer, and 5.1 μL nuclease-free water.

2.5. Statistics analysis

Real-time PCR using CT values was performed based on the factorial method to determine relative gene expression. The *18S* gene was used as the reference (normalization) gene (accession number: AF176811). The data obtained were statistically analyzed using SAS software (version 9.1) through the GLM procedure. The mean values were compared using Duncan's multiple range test to determine significant differences between treatments. A P<0.05 was considered statistically significant. The experimental design was completely randomized, and the following statistical model was used:

$$Y_{i}j = \mu + T_{i} + e_{i}j$$

Where: $Y_{i}j$ is the observed value of the i-th treatment in the j-th replication, μ is the overall mean, T_{i} is the effect of the i-th treatment, and e_{ij} is the residual error.

3. Results

Findings of this study illustrated that the supplementation of omega-3 and TRP significantly modulated the relative expression of key genes associated with the ECS in the AT of transition dairy cows. The effects varied depending on the gene and the type of supplementation, as shown in Tables 1 and 2.

Our findings revealed that *CNR1* gene expression remained unaffected by omega-3, TRP, or the interaction between omega-3 and TRP on days 21 and 42 postpartum. In contrast, *CNR2* gene expression was significantly influenced by the interaction between omega-3 and TRP. Notably, cows given omega-3, TRP, or a mixture of these two supplements showed a significant reduction in CNR2 expression (P<0.05). In relation to MGLL, the relative expression of this gene was affected by the interaction between omega-3 and TRP on days 21 and 42 postpartum. Cows that received omega-3 and TRP showed a significant increase in *MGLL* expression (P<0.05). The relative expression of the *NAAA* and *FAAH* genes was similarly influenced by the interaction between omega-3 and TRP on days 21 and 42 postpartum, and we observed a significant increase in *NAAA* and *FAAH* expression in response to omega-3 and TRP (P<0.05). Expression of the *NAPEPLD* gene, was significantly reduced (P<0.05) in cows supplemented with omega-3 on days 21 and 42 postpartum, whereas TRP alone or its interaction with omega-3 had no significant effect. Unlike other *ECS*-related genes, *COX-2* expression remained unchanged following supplementation with omega-3, TRP, or their interaction, suggesting that

Table 1. Effe^{c}t of experimental treatments on the relative expression of genes involved in the ECS in holstein dairy ^{c}ows on day 21 postpartum

Treatment	**Relative Gene Expression**						
Omega-3 (gr/cow)	***MGLL***	***CNR2***	***NAAA***	***COX2***	***NAPEPLD***	***FAAH***	***CNR1***
A	1/17^{b}	1/17^{a}	4/43^{b}	1/73^{a}	4/14^{a}	2/26^{b}	1/10^{a}
B	6/41^{a}	0/40^{b}	14/74^{a}	0/84^{a}	1/34^{b}	10/28^{a}	1/13^{a}
TRP (gr / cow)							
C	1/73^{b}	0.73^{a}	4.92b	1.39^{a}	1.67^{a}	4.28^{b}	2.65^{a}
D	4/84 ^{a}	0.83^{a}	14.25 ^{a}	1.18^{a}	1.81^{a}	8.26^{a}	2.59^{a}
Omega-3 * TRP							
AC	1/01^{d}	2/01^{a}	1/58^{d}	1/71^{a}	1/05^{a}	1/22^{d}	1/07^{a}
BC	7/90^{c}	0/84^{b}	4/35^{c}	1/76^{a}	0/31^{a}	3/02^{c}	1/03^{a}
AD	20/19^{b}	0/41^{b}	6/62^{b}	1/07^{a}	1/84^{a}	6/44^{b}	1/82^{a}
BD	29/12^{a}	0/73^{b}	20/69^{a}	0/60^{a}	1/86^{a}	14/85^{a}	1/35^{a}
P							
Omega-3	</01	</01	</01	0/17	0/01	</01	0/34
TRP	0/01	0/59	</01	0/74	0/70	0/01	0/96
Omega-3*TRP	0/01	0/01	0/01	0/67	0/76	0/01	0/51

Note: A: Level 0 omega-3; B: Level 100 omega-3; C: Level 0 TRP; D: Level 100 TRP; AC: Level 0 omega-3 * level 0 TRP; BC: Level 100 omega-3 * level 0 TRP; AD: Level 0 omega-3 * level 100 TRP; BD: Level 100 omega-3 * level 100 TRP. Values with different superscripts (a, b, c, d) are significantly different ($P<0.05$).

these dietary interventions do not influence inflammatory pathways mediated by COX-2 in AT.

4. Discussion

The transition period in dairy cattle marks a critical physiological stage, characterized by extensive metabolic, hormonal, and immune changes. During negative energy balance, dairy cows mobilize fat from AT to meet the energy demands of lactation. Activation of the ECS during negative energy balance is a natural adaptive response to stimulate feed intake and facilitate fat mobilization. The ECS is considered one of the key molecular pathways regulating energy homeostasis, appetite, lipogenesis, and lipolysis in mammals. Previous studies have shown that over-activation of the ECS can result in increased fat synthesis, impaired lipolysis, increased inflammation, and disrupted metabolic function in AT and the liver [24]. Managing systemic inflammation and negative energy balance during this phase is particularly challenging, as both can significantly compromise reproductive performance [25]. In vivo studies on transition cows supplemented with omega-3 fatty acids showed a moderate decrease in ECS activity in the blood, AT, and liver. This downregulation was linked to improved overall insulin sensitivity [2]. Modulating the ECS through dietary interventions can influence energy metabolism pathways. Omega-3 supplementation has been shown to improve insulin sensitivity and reduce lipolysis [26], while TRP affects appetite regulation and energy homeostasis [27]. The results of this study indicate that dietary supplementation with omega-3 fatty acids and TRP did not influence the expression of *CNR1* on days 21 and 42 postpartum (Tables 1 and 2). However, supplementation with omega-3 alone, as well as the interaction between omega-3 and TRP, significantly reduced *CNR2* expression on days 21 and 42 postpartum. These findings align with previous research by Dirandeh et al. (2020), which reported that AT mRNA abundance of *CNR2* and *NAPEPLD* was lowest in cows with low body condition score (BCS) loss compared to those with moderate or high BCS loss during the same postpartum period [20].

Table 2. Effect of experimental treatments on the relative expression of genes involved in the ECS in holstein dairy cows on day 42 postpartum

Treatment	Relative Gene Expression						
Omega-3 (gr / cow)	***MGLL***	***CNR2***	***NAAA***	***COX2***	***NAPEPLD***	***FAAH***	***CNR1***
A	4/46^{b}	1/43^{a}	1/95^{b}	1/73^{a}	2/03^{a}	2/12^{b}	2/05^{a}
B	24/66^{a}	0/37^{b}	7/72^{a}	0/84^{a}	0/85^{b}	10/64^{a}	2/58^{a}
TRP (gr / cow)							
C	10/60^{b}	1/21^{a}	2/10^{b}	1/39^{a}	1/45^{a}	3/83^{b}	2/95^{a}
D	18/50^{a}	1/29^{a}	5/57^{a}	1/18^{a}	1/43^{a}	8/9^{a}	2/69^{a}
Omega-3 * TRP							
AC	1/11d	1/11^{a}	1/04d	1/71^{a}	1/01^{a}	1/22^{c}	1/10^{a}
BC	7/22^{c}	0/22^{b}	7/81^{b}	1/76^{a}	0/27^{a}	3/30^{c}	1/40^{a}
AD	15/36^{b}	0/35^{b}	8/80^{b}	1/07^{a}	1/32^{a}	7/35^{b}	1/20^{a}
BD	21/45^{a}	0/45^{b}	20/69^{a}	0/60^{a}	1/35^{a}	13/22^{a}	1/07^{a}
P							
Omega-3	</01	0/04	</01	0/17	0/03	</01	</31
TRP	</01	0/87	0/03	0/74	0/97	</01	0/61
Omega-3* TRP	0/01	0/01	0/01	0/67	0/91	0/01	0/71

Note: A: Level 0 omega-3; B: Level 100 omega-3; C: Level 0 TRP; D: level 100 TRP; AC: Level 0 omega-3 * level 0 TRP; BC: Level 100 omega-3 * level 0 TRP; AD: Level 0 omega-3 * level 100 TRP; BD: Level 100 omega-3 * level 100 TRP. Values with different superscripts (a, b, c, d) are significantly different (P<0.05).

Given that *CNR2* expression is upregulated in acute or chronic inflammatory conditions [28], its downregulation in response to omega-3 supplementation may reflect the anti-inflammatory properties of these fatty acids. Omega-3s are known to suppress pro-inflammatory cytokine production [29], and to enhance antioxidant activity, which could explain the observed modulation of CNR2. The potential benefits of omega-3 supplementation extend beyond gene expression, as Nazari et al. (2019) demonstrated that improved antioxidant status in Holstein dairy cows was associated with normal luteal function, earlier resumption of cyclicity, reduced pregnancy loss, and higher conception rates. Thus, the reduction in *CNR2* expression observed in this study may contribute to improved reproductive outcomes by mitigating inflammatory responses during the postpartum period [30].

The gene expression analysis revealed that the interaction supplementation of omega-3 and TRP generated the most marked alteration in *ECS*-related genes expression including *MGLL*, *NAAA*, and *FAAH* on day 21 and day 42 of postpartum (Tables 1 and 2). The interaction of omega-3 and TRP significantly increased *MGLL*, *FAAH*, and *NAAA* expression on days 21 and 42 postpartum (Tables 1 and 2). These enzymes play critical roles in ECS regulation. MGLL is a widely present enzyme that breaks down monoacylglycerols (MGs) into free fatty acids and glycerol. It plays a role in energy balance by helping release stored fat and by degrading the endocannabinoid 2-Arachidonoylglycerol (2-AG) [28]. 2-AG and anandamide are endogenous cannabinoids that bind to and activate CNR1 and CNR2 receptors. Their signaling is terminated through enzymatic breakdown—anandamide is primarily metabolized by FAAH, while 2-AG degradation is largely mediated by MGLL [32]. It has been shown that FAAH activity is inversely related to anandamide levels, and its inhibition has been shown to reduce food intake and body weight in diet-induced obesity models [33]; this highlights *FAAH* as a key regulator of energy balance, where its modulation could help manage metabolic stress in transition cows.

Bonsale et al., (2018) reported that, compared to healthy cows, those with subclinical endometritis exhibited decreased mRNA expression of *NAAA* and *FAAH* ($P<0.05$) but increased expression of *NAPEPLD* [21], while supplementing cows' diets with omega-3 and TRP in our study showed a significant decline in expression of *NAPEPLD*. The results of this study suggest a potential synergistic interaction, where the two supplements converge on shared or complementary molecular targets within the ECS to amplify their benefits. Omega-3 fatty acids are known to have anti-inflammatory and lipid-modulatory effects, partly through their antagonistic action on CNR1 receptors and suppression of inflammatory mediators [34]; likewise, TRP, as a precursor of serotonin and kynurenine pathway metabolites, may modulate ECS activity through neuroendocrine and immune pathways [35]. Notably, the upregulation of the *NAAA*, *FAAH*, and *MGLL* genes indicates that negative energy balance and inflammation can be partially corrected by the addition of omega-3 and TRP. The study by Sina et al., (2018) illustrated a link between inflammatory status and variations in luteal activity, ovulation, and reproductive performance in early-lactation Holstein dairy cows. Inflammatory activation was associated with reduced luteal size, impaired growth of the dominant follicle, lower estradiol and progesterone concentrations, delayed ovulation, and altered luteal phase duration [36]. Thus, by modulating *ECS*-related genes, omega-3 and TRP may indirectly support reproductive recovery.

On the one hand, given the role of CNR1 in stimulating appetite, excessive suppression of ECS could potentially reduce feed intake. Therefore, maintaining a balanced modulation—rather than complete inhibition—of ECS is crucial. Omega-3 fatty acids, with their anti-inflammatory properties, and their interaction with TRP, which influences neuropeptide pathways, may help modulate ECS activity without compromising appetite. On the other hand, findings from this study revealed that supplementation with omega-3 and TRP did not cause significant alterations in the expression of *COX2* in any of the groups on days 21 and 42 postpartum. This observation indicates that although nutrition is known to modulate ECS activity, supplementation with omega-3 and TRP alone under these conditions does not appear to be an effective approach for modulating the expression of these two genes in dairy cows' AT. Failure to respond could be due to the complex interplay of metabolism during the transition, supplementation timing, and dose. Alternatively, it is possible that these compounds need to be supplemented in combination with other nutritional or management variables to cause more significant impacts on the ECS.

5. Conclusion

Overall, the findings of this study demonstrate that simultaneous supplementation with omega-3 fatty acids and TRP in transition dairy cow diets can effectively upregulate *FAAH*, *MGLL*, and *NAAA*, and downregulate *CNR2*, and *NAPEPLD* gene expression in AT. These findings underscore the potential of targeted dietary interventions to modulate the ECS in transition cows, offering a promising approach to mitigate metabolic and inflammatory challenges. By optimizing ECS activity, omega-3 and TRP supplementation could serve as part of a nutritional strategy to enhance health, productivity, and fertility in high-yielding dairy cattle during this critical period.

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Compliance with ethical guidelines

The study was conducted following the ethical guidelines of Sari Agricultural Sciences and Natural Resources University, and all animal handling procedures were carried out under institutional approval. However, at the time of the study, a formal ethics approval code was not assigned by the committee. We confirm that all procedures were performed in accordance with approved institutional standards for animal care and use.

Data availability

The data produced and/or analyzed during the present study can be obtained from the corresponding author upon request.

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Authors' contributions

Conceptualization and study design: Essa Dirandeh, Zarbakht Ansari-Pirsaraei, William W. Thatcher, and Hassan Sadri; Data acquisition: Mansoureh Ghorbanalinia; Data analysis, interpretation, administrative, technical, and material support: Essa Dirandeh; Writing: Mansoureh Ghorbanalinia and Essa Dirandeh.

Conflict of interest

The authors declared no conflict of interest.

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