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EFFECT OF FEED SUPPLEMENTATION WITH PLANT-DERIVED OMEGA 3 ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF FEMALE RABBITS

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ABSTRACT

Rabbit farming faces significant challenges due to high mortality and culling rates among females, mainly attributed to intensive reproductive rhythms, which lead to a negative energy balance and reduced fertility. Recent studies are therefore focusing on improving both the productive and reproductive performance of rabbits while also promoting their welfare. In this context, this study aims to investigate the combined effects of extruded linseed and algae *Padina pavonica* extract as dietary sources of omega-3 on female rabbits' productive and reproductive performance. Thirty-six nulliparous New Zealand White female rabbits were divided into three experimental groups (n=12) according to different diets: commercial diet (CNT group), commercial diet enriched with 5% extruded linseed alone (L5% group), and in combination with 0.2% algae *Padina pavonica* extract (L5%PP group). The rabbits were monitored from artificial insemination until weaning of the rabbit kits, and different productive and reproductive parameters were evaluated. Our results indicate that dietary enrichment with extruded linseed and alga *Padina pavonica* did not affect feed intake or live weight of female rabbits, suggesting the absence of adverse effects and validating their palatability. Additionally, no significant differences were observed in litter size, litter weight or milk yield. Interestingly, perinatal and pre-weaning mortalities were significantly lower in litters born to females of both the supplemented groups.

Key words: Algae, Fertility, Linseed, Omega-3, *Oryctolagus cuniculus*

INTRODUCTION

Rabbit farming relies on frequent breeding, and its success critically depends on the reproductive performance of female rabbits. Nevertheless, in female rabbits, intensive reproductive rhythms often induce a negative energy balance, ultimately leading to a reduction in their fertility. Primiparous rabbit does are particularly vulnerable to this negative energy balance, as their feed intake capacity and growth are not fully developed (Castellini et al., 2010). Furthermore, the energy deficit is closely associated with the reduced lifespan of reproductive females, as the primary reasons for culling in rabbit breeding are infertility and

poor body conditions (Menchetti et al., 2019). Therefore, recent studies prioritize enhancing both productive and reproductive performance as well as the welfare of this species (Menchetti et al., 2019). To achieve these goals, the scientific community is investigating the potential benefits of incorporating various nutraceuticals into the rabbits' diet. Among the investigated nutraceuticals, those containing high levels of omega-3 polyunsaturated fatty acids (n-3 PUFA) have demonstrated the most interesting results due to their numerous physiological functions (Castellini et al., 2010). This study investigates the combined effects of extruded linseed and algae *Padina pavonica* extract, as sources of n-3 PUFA, on the reproductive and productive performance of nulliparous female rabbits.

MATERIALS AND METHODS

Animals and experimental design

Thirty-six nulliparous New Zealand White female rabbits, aged 4 months, were individually housed in conventional cages (L×W×H: 75×38×25 cm) under controlled environmental conditions in a commercial farm located in Central Italy. The rabbits were randomly assigned to three groups (n=12/group) according to different diets: commercial diet (CNT group), commercial diet enriched with 5% extruded linseed alone (L5% group), and in combination with 0.2% algae *Padina pavonica* extract (L5%PP group). Following a nutritional adaptation period of 60 days, the female rabbits were artificially inseminated (AI) using fresh heterospermic semen. Ovulation was induced by intramuscular injection of 10 µg of synthetic GnRH immediately before AI. Twelve days after AI, pregnancy was diagnosed by abdominal palpation. During the experimental period (AI until weaning), the feed intake was registered daily, while live weight (LW) was recorded weekly until day 21 *post-partum*. The following reproductive and productive parameters were evaluated: receptivity, fertility, litter size at birth, litter size at weaning, litter weight at birth, litter weight at weaning, perinatal and pre-weaning mortality, as well as milk yield.

Chemical Analyses

The experimental diets were isoenergetic and formulated according to current recommendations for female breeding rabbits. The proximate chemical composition of the diets (Table 1) was determined following the AOAC methods (AOAC, 2016).

Table 1: Chemical composition of the experimental diets.

g per 100 g of dry matter	Diets ¹		
	CNT	L5%	L5%PP
Dry Matter	89.34	89.49	89.94
Crude Protein	17.72	18.33	18.59
Ether Extract	3.95	6.21	5.22
Ash	7.99	7.69	8.21
NDF	43.24	39.65	42.40
ADF	27.90	25.72	25.53
ADL	7.76	7.83	7.22

¹CNT: Commercial control diet; L5%: CNT diet supplemented with 5% extruded linseed; L5%PP: CNT diet supplemented with 5% extruded linseed and 0.2% algae *Padina pavonica* extract. NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin, following Van Soest and Wine, 1967.

The fatty acid profile of raw materials (extruded linseed and algae *Padina pavonica*) and diets (Table 2) was analyzed using GC-FID (Varian 4500). Fatty acid methyl esters were used to identify the different fatty acids. The algae *Padina pavonica* revealed a greater prevalence of saturated fatty acids primarily attributed to palmitic (C16:0) and oleic (C18:1) fatty acids. Conversely, extruded linseed exhibited higher concentration of α-linolenic acid (ALA). In terms of the fatty acid profiles of the experimental diets, both L5% and L5%PP diets exhibited higher concentrations of n-3 PUFA compared to the CNT diet. Notably, the main PUFA fraction in the CNT diet was omega-6, primarily composed of linoleic acid (LA). The L5%PP diet exhibited minimal differences in fatty acid composition compared to the L5%

diet, mainly observed in the presence of long-chain fatty acids (0.13% EPA, 0.05% DPA, and 0.06% DHA).

Table 2: Fatty acids profile (% of total fatty acids) of raw materials (extruded linseed and algae *Padina pavonica*) and diets.

Fatty acid (% of total fatty acids)	Raw material		Diets ¹		
	Linseed	Algae	CNT	L5%	L5%PP
C16:0	5.97	27.95	13.92	11.14	11.65
C18:0	4.54	12.52	3.07	3.82	3.48
C18:1	20.93	25.84	25.39	23.89	23.98
C18:2cis n-6, LA	15.11	9.72	47.63	32.41	33.53
C18:3 n-3, α -ALA	52.19	8.98	6.53	23.25	22.63
C20:5n-3, EPA	0.00	0.03	0.00	0.00	0.13
C22:5n-3, DPA	0.00	0.00	0.00	0.00	0.05
C22:6n-3, DHA	0.00	7.63	0.00	0.00	0.06

LA: linoleic acid; ALA: α -linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid. ¹CNT: Commercial control diet; L5%: CNT diet supplemented with 5% extruded linseed; L5%PP: CNT diet supplemented with 5% extruded linseed and 0.2% algae *Padina pavonica* extract.

Statistical Analysis

Kruskal Wallis test was used to compare feed intake among groups, while Linear Mixed models were used to analyse LW, milk yield, and litter size, including days after AI or after parturition as repeated factors. The models evaluated the main effects of time, group, and their interaction. The number of rabbit kits was included in the models as a covariate when appropriate. Sidak adjustment was used to conduct multiple comparisons. Perinatal and pre-weaning mortality were analysed by Generalized Linear models using Poisson distribution and Log link function. The receptivity and fertility were analysed by Chi-square, Fisher exact, and z-tests. Statistical analyses were performed with SPSS Statistics (IBM, SPSS Inc., Chicago, IL, USA). Differences were declared assuming an alpha value of 0.05.

RESULTS AND DISCUSSION

To the best of our knowledge, this is the first study to examine the combined effects of extruded linseed and the extract of *Padina pavonica* algae, as sources of n-3 PUFA, on the reproductive and productive performance of nulliparous female rabbits. Feed intake did not differ among groups during pregnancy, suggesting that both linseed and algae did not negatively affect the diets' palatability. The LW of female rabbits was affected only by time ($P < 0.001$). Specifically, during pregnancy, LW progressively increased, while during lactation it decreased within one week after parturition (data not shown). Concerning the rabbit's sexual receptivity, while the L5% and L5%PP groups demonstrated a higher percentage of does with red vulvas (75%) than the CNT group (58.8%), analysis revealed only trend towards significance ($p = 0.086$). A comparable outcome was observed regarding the rabbits' fertility, as both L5% and L5%PP experimental groups exhibited higher pregnancy rates (83.3%) compared to the CNT group (66.7%), even though statistical analysis could not find significant differences among groups ($p = 0.447$). As regards the productive parameters, no significant differences were observed in litter size among groups, neither at birth nor weaning (Table 3). Moreover, litter weight was influenced by litter size ($p < 0.01$) but not by the group. Our results are consistent with previous studies (Rebollar et al., 2014) which found no significant effects of n-3 PUFA supplementation on both litter size and weight at birth and weaning. However, our study revealed that both supplemented groups had significantly lower perinatal ($P < 0.001$) and pre-weaning ($P < 0.05$) mortality (Table 3). It is suggested that dietary n-3 PUFA supplementation during pregnancy promotes early neuronal development and regulates neurochemical aspects related to stress response, growth, and cognitive functions, being very important for the vitality of newborns, especially in the first days of life (Bernardi et al., 2012). Newborn mammals can also benefit from n-3 PUFA supplementation through milk consumption, as the fatty acid composition of milk typically reflects the composition of the

mother's diet (Rodríguez et al., 2018). Although milk composition was not assessed in our study, milk yield increased until day 18 *post-partum* ($P<0.001$) and was influenced by litter size ($P<0.001$) but without differences among groups.

Table 3: Productive performance of the experimental groups.

Parameter	Experimental groups ¹			P value
	CNT	L5%	L5%PP	
Litter size at birth (total n)	7±1	8±1	7±1	0.576
Litter size at weaning (n)	6±1	7±1	7±1	0.507
Litter weight at birth (g) ^{*†}	427±25	440±20	468±19	0.394
Litter weight at weaning (g) ^{*†}	4987±199	4814±188	4965±177	0.784
Perinatal mortality (%) [*]	6.67c±0.86	2.78b±0.56	0.00a±0.00	<0.001
Pre-weaning mortality (%) [*]	5.56c±0.79	0.00a±0.00	3.33b±0.53	0.016
Milk yield (g) [*]	161.9±2.8	158.6±2.6	159.90±2.5	0.679

Data are expressed as mean ± standard errors. ^{*} Estimated. [†] Litter size was included as covariate in the models. Values followed by the same letter in each row do not differ significantly ($P<0.05$). ¹CNT: Commercial control diet; L5%: CNT diet supplemented with 5% extruded linseed; L5%PP: CNT diet supplemented with 5% extruded linseed and 0.2% algae *Padina pavonica* extract.

CONCLUSIONS

Our data suggest that dietary supplementation with n-3 PUFA in nulliparous rabbit does: (1) did not significantly affect feed intake or live weight, suggesting no negative effects on the diets' palatability; (2) showed a tendency to enhance receptivity and fertility, although a larger sample size is needed to statistically confirm this trend; (3) reduced perinatal and pre-weaning mortality in rabbit kits. Based on these findings, dietary n-3 PUFA supplementation with extruded linseed and *Padina pavonica* algae appears to be a promising strategy for improving the productive and reproductive performance of nulliparous female rabbits.

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