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**THE IMPLEMENTATION AND EVALUATION OF A
NUTRITION EDUCATION PROGRAMME FOR
UNIVERSITY ELITE ATHLETES**

**EFFICACIA E TOLLERABILITÀ DI UN
NUTRACEUTICO NEL TRATTAMENTO DEL
PHOTO-AGING CUTANEO**

**UTILIZZO DI UN PROTOCOLLO DIETETICO
MULTIFASICO NEL TRATTAMENTO DEL
SOVRAPPESO-OBESITÀ CORRELATI ALLA
SINDROME METABOLICA**



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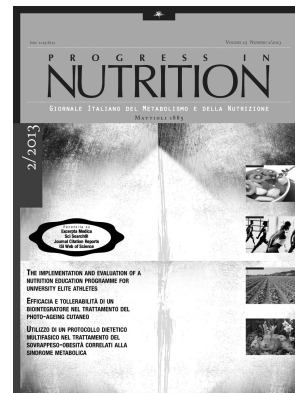
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Sommario



HIGHLIGHTS

- 71 *L. Martinelli*
The implementation and evaluation of a nutrition education programme for university elite athletes

REVIEW

- 81 *G. Zicari, V. Soardo, D. Rivetti*
La gestione delle deiezioni zootecniche: il rischio chimico

LAVORI ORIGINALI

- 90 *A. Coacci, B. Palmieri*
Efficacia e tollerabilità di un nutraceutico in formulazione perle nel trattamento del photo-aging cutaneo. Studio-pilota
- 99 *G. Meineri, G. Masoero, G. Forneris*
Use of medium chain fatty acids and a phytocomplex in diets for rabbit does
- 107 *A.L. Martinez Piñeiro, S. Ballali, I. Baldi, G. Vecchio, M. del Socorro del Rio Angulo, R. Stanchi, D. Gregori*
Subsidizing children with breakfast can help in reducing malnutrition harms: the experience of the Monte Albán community intervention program of AVSI in Mexico
- 116 *L. Vigna, C.E. Barberi, A.S. Tirelli, D. Sommaruga, L. Riboldi*
Utilizzo del protocollo dietetico multifasico (Dietacare® Named) in donne lavoratrici e sua efficacia nel trattamento del sovrappeso-obesità correlati alla sindrome metabolica

ALIMENTI – ALIMENTI INTEGRATI

126 *F. Cioni*

Le esperienze in ambito oncologico sulla Sindrome da Fatica
Cronica come modello di riferimento nel trattamento
generale della stanchezza

LETTER TO EDITOR

133 *C. Maioli, R. Paroni, G. Cighetti*

Comment on JVGA Durnin and J.Womersley article

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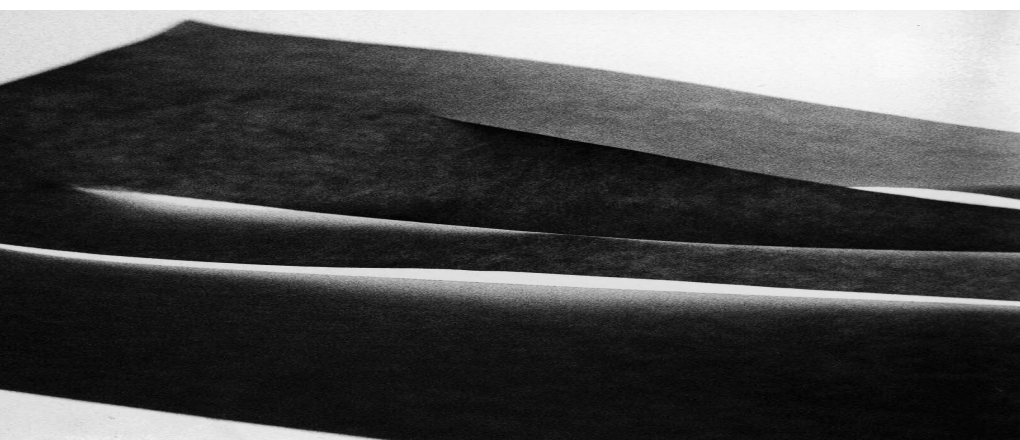
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Use of medium chain fatty acids and a phytocomplex in diets for rabbit does

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TITLE

Utilizzo di acidi grassi a media catena e di un fitocomplesso nelle diete per coniglie allattanti

KEY WORDS

Rabbit does, suckling rabbits, antibiotics, Medium chain fatty acid, Probiotan[®]

PAROLE CHIAVE

Coniglie, coniglietti lattanti, antibiotici, acidi grassi a media catena, Probiotan[®]

Summary

In the present work, four diets for lactating female rabbits have been compared: diet C, control diet; diet A, containing a "blend" of medium chain fatty acids (Biotrade S.N.C.); diet P, containing extracts plants oil (Glycine S., Menta P., Malus P., Centaurium U.) (Probiotan[®], by Biorama S.A.S.); diet M, diet containing three antibiotics (Colistine, Avylamicine, Tiamuline) used for the prevention of diseases. The 4 diets were administered to 63 female rabbits that had given birth to 452 pups, alive at 3 days post partum. The purpose of this study was to determine the survival rate of suckling rabbits. A highly significant ($P: 0.002 \div <0.0001$) reduction in mortality of pups belonging to female rabbits included in the three treatment groups (A: 16.3%, P: 17.2%, M: 14.8%) compared to puppies belonging to female rabbits included in the control group (C: 35.6%) was observed for the period between 0 and 19 days post partum. It was concluded that the vitality of the suckling rabbits in the period from 3 to 19 days post partum was significantly improved through the use of medium chain fatty acids (MCFAs) and by Probiotan[®] integration in the diets of their mothers. This study shows that the MCFAs and plant extracts may therefore be considered as alternative remedies to antibiotics, as they are free of side effects and do not induce antibiotic resistance in rabbits.

Riassunto

Nel presente lavoro, sono state confrontate 4 diete per coniglie in lattazione: dieta di controllo C; dieta A, contenente una "miscela" di acidi grassi a catena media (Biotrade S.N.C.); dieta P, contenenti estratti oleosi di piante (Glycine S., Menta P., Malus P., Centaurium U.; Probiotan[®], Biorama S.A.S.); dieta medicata M, contenente 3 antibiotici (Colistina, Avylamicina, Tiamulina) usati per la prevenzione delle malattie. Le diete sono state somministrate a 63 femmine di coniglio, le cui nidiate erano composte da 452 cuccioli vivi a distanza di 3 giorni dal parto. Lo scopo di questo studio era di determinare il tasso di sopravvivenza dei conigli neonati. È stata osservata una riduzione altamente significativa ($P: 0.002 \div <0.0001$) della mortalità dei coniglietti nati dalle madri appartenenti ai 3 gruppi trattati (A: 16,3%; P: 17,2%; M: 14,8%) rispetto dei coniglietti

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nati dalle madri appartenenti al gruppo di controllo (C: 35,6%) nel periodo compreso fra 0 e 19 giorni post partum . La vitalità dei neonati è significativamente migliorata attraverso l'utilizzo di acidi grassi a catena media (MCFAs) e attraverso l'integrazione con Probiotan®, nelle diete delle coniglie allattanti. Questo studio dimostra che gli acidi grassi a media catena e i fitocomplessi, utilizzati a scopo profilattico, possono essere considerati come rimedi alternativi agli antibiotici, poiché sono privi di effetti collaterali e non inducono antibiotico-resistenza.

Introduction

The rabbit rearing is an important sector of animal production in Italy, because of some peculiar features that distinguish these animals: high reproductive potential, rapid growth, short generation interval and highly appreciated meat quality. In recent years, intensive rabbit rearing, has reached a very critical point that is characterized by high mortality of the suckling rabbits in the pre-and post-weaning periods (1). This high mortality rate among the litters depends mainly on the health and nutrition of the lactating female rabbits, and their ability to produce adequate amounts of milk (2, 3). In fact, the nutrition of the suckling rabbits completely depends on the availability of mother's milk during the first 20 days of life (4). In lactation period, female rabbits have high energy requirements and are often not able to meet these nutritional needs through diet.

Therefore, insufficient consumption of food and energy of the female rabbits can have drastic ef-

fects on milk production and on the assumption of milk intake by suckling rabbits (5, 6). The high energy requirements of females rabbits can be satisfied through an increase in the starchy component of the rations. However, the problems resulting from the intake of starch, whose components undigested can serve as substrate for the growth of potentially pathogenic intestinal strains, should be taken into account (7). An insufficient milk supply can often cause enteropathy in the suckling rabbits which can result in the severe loss of litters. The milk, in fact, is important for the prevention of intestinal disorders; this is due to the bacteriostatic role of the medium chain fatty acids (C8 and C10) which are prevalent in rabbit milk and are liberated by the gastric lipase of suckling rabbits (8-10). Currently, many nutritional approaches are adopted in the rearing of rabbits to protect the health and milk production of lactating female rabbits and, thus, indirectly, to ensure the survival of the lit-

ters. The strategies to reduce the incidence of severe enteric diseases, which penalize the productivity of intensive rearing, are obtained through the addition of organic acids, herbal extracts, enzymes, prebiotics and probiotics to the feedstuffs (11, 12). Supplementation with these substances has the aim of reduce and eliminate the use of antibiotics, as a preventive remedy. The purpose of this study was to compare different interventions of integration of the diets of lactating female in rabbits to evaluate the survival rate of suckling rabbits and to assess the production parameters of the litters. The supplementation with these substances has the aim of reducing and eliminating the use of antibiotics as a preventive remedy, as they are harmful to animal and human health. The purpose of this study was to compare different dietary interventions on female rabbits in order to evaluate survival rate of the their suckling rabbits and to evaluate the productive parameters of the litters.

Materials and methods

Diets and animals

Four diets for lactating female rabbits were formulated:

- C-Control: standard diet;
- A-MCFAs: standard diet supplemented with a blend of medium chain fatty acids (MCFAs by BIOTRADE S.N.C., Mirandola, MO, IT) (C8-C10) at a dose of 0,5% to replace an equal amount of soybean oil;
- P-Probiotan: standard diet with the addition of vegetable oil extracts (Probiotan® by BIORAMA S.A.S., Rogeno, LC, IT) at a dose of 1% to replace an equal amount of soybean oil;
- M-Medicated: standard diet with the addition of antibiotics at the therapeutic dose (Colistine 180 ppm, Avylamicine 60 ppm, Tiamuline 40 ppm);

Four groups of female rabbits (63 pluriparous lactating female rabbits) of *Macchiata italiana* and *Bianca Italiana* strains, derived from an Italian ANCI selection scheme, were submitted to an extensive reproductive rhythm. The litters were weaned at 40 days. The rabbits received the 4 diets during the *post partum* (p.p.) and the diets and water were provided *ad libitum*. The rabbits were weighed on the third day (d.) post-partum and on the nineteenth day p.p. The parameters of vitality of suckling rabbits were

measured between 3 and 19 d. p.p. In addition, some zootechnical parameters of rabbits females and litters were evaluated: maternal and litter weight on the third day and on the nineteenth day p.p., furthermore the index of food consumption of rabbits was examined.

Statistical analysis

The mortality rate of the suckling rabbits was elaborated by using three tests: 1) the Fisher's exact method; 2) the SAS PROC. CATMOD (13) separately for a bimodal response (suckling rabbits dead/suckling rabbits and 3) the SAS PROC. CATMOD (13) separately for a tetramodal response which considered the appearance of the mortality within litter (total, partial or null) considering four classes of suckling rabbits (suckling rabbits dead in all-dead litter/suckling rabbits dead in part-dead litter/suckling rabbits alive in part-alive litter/suckling rabbits alive in all-alive litter) within each treatment.

Results

Table 1 reports the ingredients of the four diets (C, A, M and P) administered to the lactating female rabbits. Table 2 shows the chemical composition of the four studied diets.

A total of 63 female rabbits, with 452 suckling rabbits, alive at 3 days p.p., were controlled (Table 3). In comparison with the control group (C: 35.6%), a highly significant ($P: 0.002 \div <0.0001$) reduction in suckling rabbits mortality was found in the three treated groups (A, 16.3%, P, 17.2% M, 14.8%; Table 3). In Tables 4 and 5 are reported the zootechnical parameters of the female rabbits and of the total litters: the weight of the lactating female rabbits and of the litters at 3 days and at 19 days *post partum*; the consumed feed and consumption index of the lactating female rabbits. The number of suckling rabbits, alive at 19 d. p.p. and the litters weight (g) at 19 d. p.p. were highly significant (<0.00), according to the statistical model (1Cov) (Table 4) No effect of the experimental diet or breed factors was statistically significant on the parameters examined (Table 5). Table 1 reports the ingredients of the four diets (C, A, M and P) administered to the lactating female rabbits. Table 2 shows the chemical composition of the four studied diets. A total of 63 multiparous female rabbits with 452 suckling rabbits alive at 3 days p.p. were examined (Table 3). A highly significant ($P: 0.002 \div <0.0001$) reduction in suckling rabbits mortality was found in the three treated groups (A, 16.3%, P, 17.2% M, 14.8%; in comparison with the control group (C: 35.6%) (Table 3).

Table 1 - Ingredients of the diets (%).

Ingredients	Diet C-Control	Diet A-MCFAs	Diet P-Probiotan®	Diet M-Medicated*
Corn	14.8	14.6	15	15.2
Barley	19.2	19.4	19	18.8
Dehydrated alfa alfa	45.6	45.4	46	46.2
Soybean seed	12.4	12.6	12	11.8
Soybean Oil	4	3.5	3	4
Vitamin mineral premix**	2	2	2	2
Lignosulfite	2	2	2	2
Probiotan®	0	0	1	0
Medium chain fatty acids ***	0	0.5	0	0

*M: medicated feed with Colistine 180 ppm, Avylamicine 60 ppm, Triamuline 40 ppm.

**Vitamin mineral premix of Kg diet: Vit.A 200 U.I.; alfa-tocoferyl acetate 1 mg; Niacine 72 mg; Vit B6 16 mg; Coline 0.48 mg, DL-methionine 600 mg; Ca 500 mg, P 920 mg; K 500mg, Na 1 g, Mg 60 mg, Mn 1.7 mg; Cu 0.6 mg.

*** Medium chain fatty acids: octanoic acid (C8:0), 58.2%; decanoid acid (C10:0), 41.8%.

Table 2 - Chemical composition (% DM basis) of the diets.

Diet composition	Diet C-Control	Diet A-MCFAs	Diet P- Probiotan®	Diet M-Medicated*
Dry matter (%)	92.3	92.5	91.8	93
Organic matter (%)	93.4	93.6	92.7	94
Crude protein (%)	16.5	17.2	16.4	17.3
Crude Fiber (%)	17	17.2	17	17.4
Ether Extract (%)	4.9	4.7	4.8	5
Ash (%)	6,6	6.7	6	6.4
Gross Energy (J/Kg)	17.7	17.4	17.2	18

In Tables 4 and 5 are reported the zootechnical parameters of the female rabbits and of the total litters: the weight of the female rabbits and of the litters at 3 and 19 days *post partum*; the consumed

feed and the consumption index of the female rabbits. The number of suckling rabbits, alive at 19 d. p.p., and the litters weight (g) at 19 d. p.p. were highly significant (<0.00); according to the statistical

model statistical model (¹Cov) (Table 4) No effect of the experimental diet or breed factors was statistically significant on the parameters examined (Table 5).

Table 3 - Suckling rabbits mortality (3-19 days post partum) in the four group of Lactating female rabbits

Diets	Pluriparous Lactating female rabbits	Total N *	Average N *	Total N**	Average N**	*** %	Probability of the contrasts C-Control vs. A, P, M according the three tests		
C-Control	15	104	6.93	37	2.47	35.6%	Fisher ¹	CAT2 ²	CAT 4 ³
A-MCFAs	14	104	7.43	17	1.21	16.3%	0.0025	0.0019	0.0002
P- Probiotan®	17	122	7.18	21	1.24	17.2%	0.002	0.002	0.0008
M-Medicated	17	122	7.18	18	1.06	14.8%	0.0003	0.0004	<.0001
Raw Means / Total	63	452	7.17	93	1.48	22.4%			

N*: Number of suckling rabbits alive three days post partum

N**: Number of suckling rabbits dead between 3 and 19 days post partum

*** %: Suckling rabbits mortality

¹ Fisher exact test

² CATMOD test, bimodal response (suckling rabbits dead/suckling rabbits alive)

³ CATMOD test, tetramodal response (suckling rabbits dead in all-dead litter / suckling rabbits dead in part-dead litter/suckling rabbits alive in part-alive litter/suckling rabbits alive in all-alive litter)

Table 4 - Statistical analysis of the suckling rabbits and litters (L) traits.

Variables	* R ² Model	* Variation Coefficient %	* Mean Square Error	* Mean	** Group ²	** Breed ³	Cov ¹
N. alive 3 d. p.p.	0.32	33	2.2	6.75	0.93	0.81	.
N. dead 3-19 d. p.p.	0.14	154	2.3	1.48	0.43	0.47	0.03
N. alive at 19 d. p.p.	0.42	40	2.3	5.70	0.43	0.47	<.00
L .W.(g) 3 d. p.p.	0.22	25	168	681	0.91	0.11	0.00
L. W. (g) 19 d. p.p.	0.35	24	528	2240	0.88	0.40	<.00
W.(g) 3 d. p.p.	0.05	25	24	95	0.97	0.12	0.63
Average W.(g) 19 d. p.p.	0.08	20	69	342	0.96	0.30	0.12

N. alive 3 d. p.p.: number suckling rabbits alive at 3 d. p.p.; N. dead 3-19 d. p.p.: number suckling rabbits dead between 3 and 19 d. p.p.; N. alive at 19 d. p.p.: number suckling rabbits alive at 19 d. p.p.; L. W. (g) 3 d. p.p.: litters weight (g) at 3 d. p.p.; L. W. (g) 19 d. p.p.: litters weight (g) at 19 d. p.p.; W.(g) 3 d. p.p.: weight (g) of suckling rabbits at 3 d. p.p.; Average W.(g) 19 d. p.p.: average suckling rabbits weight (g) at 19 d. p.p.; * Statistics; ** Probability; Cov¹: Probability of the covariate number of suckling rabbits alive at 3 d. p.p.; Group²: the 4 diets; Breed³: Bianca italiana Vs Macchiata italiana

Discussion

The high density of animals in intensive rearing of rabbits can lead

to increased infections and especially in female rabbits and their litters was observed a high incidence of digestive disorders (14)

In the rabbit farms the antibiotics, as food additives, are used to prevent the proliferation of pathogenic agents in the digestive tract,

Table 5 - Statistical analysis of the lactating female rabbits traits.

Variables	*R ² model	*Variation Coefficient %	* Mean Square Error	*Mean	**Group ²	**Breed ³	Cov ¹
W. 3 d. p.p (g)	0.10	10	431	4349	0.26	0.17	0.54
W3-19 d. p.p. (g)	0.06	9	388	4479	0.58	0.52	0.38
Diff. W. (g)	0.09	374	319	85	0.38	0.62	0.22
F. I. L. (kg)	0.04	16	1.1	7.14	0.76	0.59	0.39
F.C.I (kg/kg)	0.20	40	1.4	3.51	0.84	0.38	0.00

W 3 d. p.p.: Lactating female rabbits weight (g) at 3 d. p.p.; *W 3-19 d. p.p.*: Lactating female rabbits weight (g) at 19 d. p.p.; *Diff. W (g)*: Difference of the Lactating female rabbits weight (g); *F. I. L. (kg)*: Feed intake of the Lactating female rabbits (kg); *F.C.I (kg/kg)*: Feed Consumption Index of the lactating female rabbits (kg/kg); * Statistics; ** Probability; *Cov¹*: Probability of the covariate number of suckling rabbits alive at 3 d. p.p.; *Group²*: the 4 diets; *Breed³*: Bianca italiana Vs Macchiata italiana

to increase the availability of nutrients and to improve the productive performance of female rabbits. The efficacy of antibiotics as growth promoters in other species, such as pigs, has been well documented, but this practice is currently forbidden in European Community also for rabbits (15). The ban on the use of antimicrobial agents, growth promoters (AGP) has increased the problems of the rabbit rearing. However, the use of medicated feeds, for therapeutic purposes, is still allowed in situations of disease, but further information about the possible side-effects of this practice are necessary (15). Moreover, the use of antibiotics at therapeutic doses in farm animals has been viewed critically because of the role of antibiotics in the development of resistant bacteria in animals and for the presence of antibiotic residues in the meat, which can compro-

mise human health (14, 16). Because of the lack of efficient alternatives (vaccines), a global approach including nutritional measures, is necessary in order to reduce the use of antibiotics. In recent years, researchers have studied some alternative substances and physiological additives, such as acidifiers, to improve the performance of rabbits (17). In our work, the addition of MCFAs to the diets has determined a positive and protective effect of the vitality of the suckling rabbits. This result confirms other studies that show that the use of MCFAs can be a powerful tool to help maintain the health of the gastro-intestinal tract of poultry and rabbits and to improve their zootechnical performances (18). The MCFAs are in fact substances that are capable of positively influencing the commensal microflora of the cecum of rabbits and may harm the

pathogenic bacteria. The MCFAs added to the feed seem to play a key role against pathogenic bacteria because they can selectively penetrate the lipid membrane of the bacterial cells through the autolytic enzymes and they can be divided into protons and anions that determine the bacterial lysis of the body (19). Furthermore, the use of MCFAs in diets seems to provide several beneficial effects to animals, such as the improvement of the coefficient of transformation, the improvement of growth performance, and the absorption of minerals (20, 21). A group of Czech researchers has studied the use of MCFAs in the diets of rabbits and has found that the inclusion of 0.5% of caprylic acid in the diets of rabbits can reduce the mortality of rabbits in post-weaning period (22). Moreover, some studies have shown that the use of MCFAs in diets may improve the

immune response (21). In addition to MCFAs acids, other supplements can be considered as alternatives to antibiotics in the poultry and rabbit rearing, for example, plant extracts, essential oils and spices, all products that have positive effects on intestinal microflora (23-27). Specific antimicrobial activity on gram negative bacteria has been demonstrated for some herbs (28). Moreover, the antioxidant properties of natural antioxidants, such as *Thymus vulgaris* or *Curcuma longa*, may decrease the reactive oxygen species and thereby decrease protein oxidation, which affects the growth and the nitrogen excretion of farm animals (19). The researches on the use of phytocomplexes concerning the performances of lactating female rabbits and the survival of suckling rabbits is very limited compared to other species; most of these studies are based on empirical trials and the results are not consistent (29). The results of many studies on plant extracts are very different: Eiben et al. (30) in a study on 510 young rabbits after weaning fed with four preventive treatments, showed a mortality rate worst in rabbits treated with plant extracts (35.4%) compared to the negative control (25). Badagliacca et al. (31) registered an average monthly mortality rate of 14.4±11% (N = 46030 growing rabbits), with use of the antibiotics

and of 29.3±18% in the control groups. In the present study, we used a supplement (Probiotan) containing extracts of vegetable oils (Glycine S., P. Menta, P. Malus, Centaurium U.). All these plants are rich in phenolic compounds (isoflavones, quercetin, epicatechin) and antioxidants. Our results have shown a significant effect of enhancing the vitality of the suckling rabbits through the addition of Probiotan and through the addition of MCFAs to the diets of lactating female rabbits compared to the standard diet. These results are not significantly different from those obtained with the use of antibiotics (M). In conclusion, MCFAs and Probiotan have shown significant and positive effects on the vitality of litters. These results are interesting because the oil extracts and the MCFAs are free from side effects and do not induce antibiotic resistance if used in the long term. However, a combination of these alternative substances may be the object of further and prolonged studies in this field.

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