

THE ROLE OF FOLIC ACID AND IRON IN REPRODUCTIVE PERFORMANCE OF NEW ZEALAND WHITE DOES AND THEIR KITS

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ABSTRACT : Forty eight lactating non-pregnant New Zealand White does 9 months of age and weighing 3468 g and in the third parity were used in the present study. The does were randomly divided into 4 equal groups of 12 each and were mated twice. The four groups of the pregnant does were given an unsupplemented diet (control) or a diet supplemented 5 mg folic acid (FA), 80 mg Fe (as Ferrous Sulfate, FeSO₄) or 5 mg FA + 80 mg Fe/kg dry matter, respectively, for a period of two parities with two lactation cycles. All treatments had increased (P<0.05) maternal weight at 14, 21 and 28 days of gestation, as compared to the control group. In does receiving FA or Fe, both litter size at birth and at weaning increased (P<0.05), while FA + Fe group insignificantly increased litter size at weaning only. Total mortality of young rabbit was significantly (P<0.05) lower in all

treatments. FA, Fe, and FA + Fe treatments increased (P<0.05) litter weight at weaning (5080, 3730 and 3160, respectively vs. 2069 g) and improved (P<0.05) kit weight at weaning (666, 598 and 638, respectively vs 559 g) compared to the control group. On day 28 of gestation, plasma progesterone level is increased in FA supplemented does and both progesterone and estradiol-17 β levels are increased in does supplemented with Fe treatment. Plasma concentrations of T₄, estradiol-17 β and progesterone were significantly higher on day 28 of gestation in does supplemented with FA + Fe. Total milk yield/doe/lactation cycle was significantly higher in all treated groups than in the control (P<0.05). It was concluded that the supplementation of FA or FA + Fe to a basal diets of pregnant does will improve their reproductive performance.

RÉSUMÉ : Rôle de l'acide folique et du fer dans les performances de reproduction de lapines Néozélandais Blanc et de leurs lapereaux.

Pour cette étude, 48 lapines Néozélandais Blanc allaitantes, non gestantes, âgées de 9 mois pesant 3468g, en troisième portée, ont été utilisées. Les lapines ont été réparties au hasard en 4 lots égaux de 12 chacun ; elles ont été saillies 2 fois. Un aliment non supplémenté (Contrôle) ou un aliment supplémenté avec 5mg d'acide folique (FA) ou 80mg de Fe (Sulfate de Fer, FeSO₄) ou 5mg FA + 80mg Fe/kg a été distribué aux quatre groupes respectifs, pour deux cycles de reproduction consécutifs. Le poids de la mère à 14, 21 et 28 jours de gestation a augmenté (P<0.05) dans tous les groupes traités par rapport au groupe contrôle. Chez les lapines recevant soit FA soit Fe, la taille de la portée à la naissance et au sevrage augmente (P<0.05) tandis que pour le groupe FA + Fe seule la taille de la portée au sevrage augmente non significativement. La mortalité

totale des lapereaux est significativement (P<0.05) plus basse pour tous les groupes traités. Comparé au groupe contrôle, le poids de la portée au sevrage augmente (P<0.05) pour les groupes FA, Fe et FA+Fe (5080, 3730 et 3160g respectivement, vs 2069g) ainsi que le poids par lapereau au sevrage (666, 598, 638g respectivement, vs 559g). Au 28ème jour de gestation le taux de progestérone plasmatique augmente dans le groupe supplémenté avec FA de même pour le taux de progestérone et 17 β -estradiol chez les lapines du lot Fe. Les concentrations dans le plasma de T₄, 17 β -estradiol et de progestérone sont significativement augmentées au 28ème jour de gestation chez les lapines recevant FA+Fe. La production laitière totale/lapine/cycle de lactation est significativement plus élevée (P<0.05) pour tous les groupes traités par rapport au groupe contrôle. On peut conclure que la supplémentation d'un régime de base avec FA ou FA+Fe pour des lapines gestantes améliore leurs performances de reproduction.

INTRODUCTION

Folates are essential as coenzymes for the synthesis of both purines and pyrimidines and accordingly are involved in the synthesis of nucleic acid (HUENNEKENS, 1968). Supplemental folic acid (FA) was shown to increase RNA, DNA and protein content of foetuses in rats (MORGAN and WINICK, 1978) and sows (TREMBLAY *et al.*, 1989). Supplementation of FA was also shown to improve survival rate in 1 to 12 day old pig embryos to prevent or reduce embryonic mortality in polytocous species (MARTINENKO, 1977), to increase live foetuses number (HARPER *et al.*, 1989) and litter size at birth (MATTE *et al.*, 1984). At the same time, iron is required in large amounts by the foetuses for bone and haemoglobin formation. In addition, Fe nutrition may alter folate utilization in maternal and neonatal pigs (O'CONNOR *et al.*, 1989) and sheep (WANG *et al.*, 1991) through its effect on the storage of folate and vitamin B₁₂ in liver or erythrocytes. Studies on the effects of FA or Fe on the reproductive performance of New Zealand does are limited and the changes in hormonal levels of pregnant does as function of these treatments have not been reported. The present study was undertaken to determine whether addition of FA, Fe or FA + Fe to the commercial basal diet would affect reproductive performance of does and growth of their kits.

MATERIALS AND METHODS

The present study was conducted in the Rabbitry of the Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The study included 48 lactating, non-pregnant, 9 month old New Zealand White does in their third parity, weighing 3468 \pm 188 g. The animals were subjected to pathologic and parasitic examinations in the Laboratories of the College of Veterinary Medicine at Zagazig. Ingredients and chemical composition of the concentrated basal diet according to AOAC (1970) which included minerals and vitamins are presented in Table 1. Average daily intake of the concentrated basal diet was restricted to 165 \pm 16g/doe. The animals were assigned randomly to four groups of 12 does each. The 1st group was fed a commercial basal diet (Table I) and considered as control. The 3 experimental groups were supplemented with respectively 5 mg FA (The Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt), 80 mg Fe as ferrous sulfate (FeSO₄) and 5 mg FA + 80 mg Fe per kg DM in commercial basal diet.

In each group, the non-pregnant does were adjusted to their treatments for 7 days prior to mating, then each doe was mated twice whenever possible. Abdominal palpation was used as a pregnancy test on day 10 after mating, and the non

Table 1 : Ingredients and chemical analysis of the concentrated basal diet* for the control and treated groups.**

Items	% of Total Diet
<i>Ingredients:</i>	
Barley	32
Berseem hay	28
Cotton seed meal	3
Soybean meal	10
Wheat bran	21
Molasses	2.85
Mixture of vitamins and minerals*	0.3
Bone meal	2.5
Sodium chloride	0.25
DL-Methionine	0.1
Total	100
<i>Chemical Analysis (% on DM Basis)</i>	
Dry matter	92.90
Organic matter	82.14
Crude protein	16.20
Crude fiber	14.40
Ether extract	1.80
Nitrogen free extract	49.74
Ash	10.76
Calculated DE (Kcal/kg diet) **	2528

* Each one kilogram of vitamins and minerals mixture contains: Vit. A 20000 IU; Vit. D₃, 150000 IU; Vit E, 8.33g; Vit. K, 0.33g; Vit. B₁, 0.33g; Vit. B₂, 1g; Vit. B₆, 0.33g; Vit. B₅, 8.33g; Vit. B₁₂, 1.7mg; Pantothenic acid, 3.33g; Biotine, 33mg; Folic acid, 0.83g; Choline Chloride, 200g; Zn, 11.7g; Fe, 12.5g; Cu, 0.5g; I, 33.33mg; Se, 16.6mg; Mg, 66.7g and Mn, 5g.

** Digestible energy as kcal / kg diet was calculated based on the DE of each ingredient in the concentrated basal diet according to CHEEKE (1987).

*** The concentrated basal diet was analyzed to contain 128.82 ppm Fe.

pregnant does were excluded. The dietary treatments were continued for two parities and lasted until the end of their lactation cycles. The does in all groups were weighed on days 0 (at mating), 7, 14, 21 and 28 of pregnancy, and the increase in maternal weight with the advance of pregnancy was calculated on days 7, 14, 21 and 28.

Litter size at birth and at weaning were recorded. Litter and kit weights were also recorded throughout the periods

studied. Percentages of stillbirths and preweaning mortality were calculated, as follows : stillbirth % = (number of dead kit x 100)/total number of kits at birth; and preweaning mortality % = (litter size at birth)-(litter size at weaning) / litter size at birth.

Milk yield measurements were estimated twice weekly for the does in all production cycles, and over all mean for each group was calculated. Estimation of this trait began from young birth until weaning. The kits were deprived of suckling for 24 hours by separation between them and their mothers. Thereafter, the kits were weighed to the nearest gram before suckling. After suckling was completed, the kits weighed again. The increase in kits weight was indicated as well as the daily milk yield in grams.

On day 28 of gestation, blood samples were collected from the ear veins of does. Plasma hormone concentrations were determined using ¹²⁵I-radio-immunoassay kits manufactured by Immunotech S.A., Marseille, France for triiodothyronine and thyroxine; ICN Biomedicals, Costa Mesa, California for estradiol-17 β and Diagnostic Products Corporation, Los Anglos, USA for progesterone.

Data of the present study were statistically analysed by ANOVA as a completely randomized design (SNEDECOR and COCHRAN, 1982) according to the following model:

$$Y_{ij} = \mu + t_i + e_{ij}$$

where,

Y_{ij} = An observation.

μ = The over all mean.

t_i = Effect of treatments.

e_{ij} = Random error.

Significant differences of means were tested using least significant difference (LSD) according to SNEDECOR and COCHRAN (1982).

RESULTS

Data presented in Table 2 show that supplementing the commercial basal diet of the pregnant does with 5 mg FA, 80 mg Fe, or FA + Fe tended to increase maternal weight, but insignificantly, at day 7 of gestation. However, at 14, 21 and 28 days of gestation, maternal weight gains were greater ($P < 0.05$) in all treated groups compared with the control group.

As shown in Table 3, addition of FA or Fe to the basal diet of pregnant does increased ($P < 0.05$) litter size both at birth and at weaning. FA only reduced ($P < 0.05$) both stillbirth

Table 2 : Effects of folic acid, iron and folic acid + iron treatments on maternal weight gain ($\bar{X} \pm SE$) of New Zealand White does.

Treatment	Number of pregnant does		Maternal weight gain during gestation (g) on days			
	n1	n2	7	14	21	28
Control	11	11	87.0 ^a \pm 6.2	149.2 ^a \pm 7.7	222.5 ^a \pm 21.4	339.4 ^a \pm 26.9
Folic Acid	12	11	102.0 ^a \pm 12.6	238.1 ^b \pm 1.2	376.6 ^b \pm 39.8	546.6 ^{cb} \pm 51.8
Iron	11	10	113.5 ^a \pm 17.3	256.1 ^b \pm 5.7	387.1 ^b \pm 55.1	577.5 ^c \pm 47.2
Folic Acid + Iron	12	11	103.5 ^a \pm 13.0	203.0 ^b \pm 9.5	289.1 ^b \pm 19.3	417.2 ^b \pm 10.4

n1 and n2 equalled the number of pregnant does in the 1st and 2nd parities of the experimental period, respectively.

a,b,c : Means in the same columns with different superscripts differed ($P < 0.05$).

Table 3 : Effects of folic acid, iron and folic acid + iron treatments on gestation length, litter size ($\bar{X} \pm SE$), stillbirth (%) and preweaning and total mortality (%) of New Zealand White does.

Treatments	Gestation length days	Litter Size(n) at Birth	Litter Size(n) at Weaning	Stillbirth %	Preweaning Mortality %	Total Mortality %
Control	32.3 ^a ± 0.2	5.7 ^a ± 0.7	4.0 ^a ± 0.8	8.06 ^a	29.82 ^a	37.88 ^a
Folic Acid	32.9 ^a ± 0.4	7.9 ^b ± 0.7	7.7 ^c ± 0.6	3.65 ^b	2.53 ^b	6.18 ^b
Iron	32.9 ^a ± 0.3	8.0 ^b ± 0.8	6.5 ^{bc} ± 0.7	3.61 ^b	18.75 ^a	22.36 ^a
Folic Acid + Iron	32.7 ^a ± 0.4	5.6 ^a ± 0.7	5.3 ^{ab} ± 0.7	6.89 ^a	1.85 ^b	8.74 ^b

a,b,c and d : Means in the same columns with different superscripts differed ($P < 0.05$). ; n : Number kits alive.

and preweaning mortality %. FA + Fe insignificantly raised litter size only at weaning and reduced ($P < 0.05$) preweaning mortality. Total mortality (stillbirth + preweaning mortality %) was to be lower by 83.6, 76.9 and 40.9% in does received FA, FA + Fe and Fe, respectively, than in control does.

Table 4 shows that litter weights significantly ($P < 0.05$) increased at birth and at 7 and 21 days of age and also at weaning in all treated groups. At day 14, litter weights were increased ($P < 0.05$) only with FA treatment. At weaning, litter weights increased 146%, 80% and 53% in response to supplementation of FA, Fe and FA + Fe, respectively to a basal diets of does, compared to non-supplemented ones. Considering kit weight at weaning in relation to different treatments, it can be seen in Table 4 kit weights at weaning were 19 and 14% higher ($P < 0.05$) in does supplemented with FA and FA + Fe, respectively than in non-supplemented ones. However, insignificant increase in kit weight at weaning (6.9%) was detected with Fe treatment.

Data presented in Table 5 show that supplementary FA to pregnant does increased ($P < 0.05$) progesterone level, and Fe also increased ($P < 0.05$) both estradiol-17 β and progesterone levels on day 28 of gestation. FA + Fe supplemented to pregnant does caused a significant increase in T4, estradiol-17 β and progesterone concentrations on day 28 of gestation. Milk yield / doe / lactation cycle is significantly increased in does receiving FA, Fe, or FA + Fe compared to control does (Table 5). The rise in approximate milk yield ranged between 55 and 48%.

DISCUSSION

The significant increase in maternal weight as a function of FA treatment is in agreement with previous results, in gilts where body weight gains during gestation

(EASTER *et al.*, 1983); and placental weight with foetal wet weight in gilts (HARPER *et al.*, 1992) and number of pig born and live pigs at birth, day 14 and day 21 (THALTER *et al.*, 1989) were increased significantly with the addition of 0.2-0.5mg FA/kg diet. This increase in maternal weight possibly was due to the physiological effect of FA on the foeto-placental development, whereby during gestation placental transfer of folates from the mother to the foetus takes place (GIRARD *et al.*, 1995). This leads to an increase in cell division (HUENNEKENS, 1968), protein synthesis (TREMBLAY *et al.*, 1989), RNA content and RNA / DNA ratio in the foetuses (MORGAN and WINICK, 1978 ; HARPER *et al.*, 1992) which is reflected by the foeto-placental and maternal weight gains as well as kit birth weights. The improvement in maternal weight gain with Fe treatment (Table 2) may be a result of the increase in foetal growth, since the transfer of Fe from maternal blood to placental tissue and from there to foetal blood may had a role in such increase. It is of relevance that the increases in maternal weight and kit weight at birth with FA + Fe treatment were coincident with increases of T4, estradiol-17 β and progesterone levels (Table 5) which probably have potential for improving tissue synthesis during embryonic period that was reflected in the kit weight at birth. Estradiol-17 β is involved in rabbit reproductivity (UBILLA and REBOLLAR, 1994) and increased average daily gain in animals because it increases insulin like growth factor I concentrations (HAYS *et al.*, 1995).

Supplemental FA had a numerically favourable effect on litter size in sows (MATTE *et al.*, 1984) and on number of live foetuses (HARPER *et al.*, 1989). The level of 5 mg FA/kg diet increased survival rate of foetuses during gestation of sows (TREMBLAY, 1988 ; TREMBLAY *et al.*, 1989). A similar trend was observed in gilts by using 4 mg FA/kg diet (STANCIC *et al.*, 1993). It can be noted that total mortality reached a

Table 4 : Effects of folic acid, iron and folic acid + iron treatments on litter and kit weights ($\bar{X} \pm SE$.) during lactation in New Zealand White does.

Treat-ments	Litter Weight (g) at					Kit Weight (g) at				
	Birth	7-days	14-days	21-days	Weaning	Birth	7-days	14-days	21-days	Weaning
Control	324 ^a ± 36	880 ^a ± 90	1346 ^a ± 121	1600 ^a ± 171	2069 ^a ± 88	58.7 ^a ± 2.7	168 ^a ± 17	258 ^a ± 24	404 ^a ± 39	559 ^a ± 41
Folic Acid	510 ^c ± 37	1440 ^c ± 79	2301 ^b ± 170	3300 ^c ± 148	5080 ^c ± 97	67.0 ^b ± 4.7	201 ^b ± 14	296 ^b ± 17	439 ^b ± 22	666 ^b ± 18
Iron	449 ^{bc} ± 27	1142 ^b ± 110	1670 ^a ± 105	2286 ^b ± 108	3730 ^b ± 89	58.2 ^a ± 2.8	157 ^a ± 6	255 ^a ± 15	359 ^a ± 28	598 ^a ± 49
Folic acid + Iron	397 ^b ± 48	1084 ^b ± 91	1780 ^{ab} ± 128	2366 ^b ± 103	3160 ^b ± 80	79.6 ^b ± 10	218.6 ^b ± 19	343 ^b ± 25	462 ^b ± 28	638 ^b ± 50

a,b,c: Means in the same columns with different superscripts differed ($P < 0.05$).

Table 5 : Effects of folic acid, iron and folic acid + iron treatments on serum hormonal levels on day 28 of gestation and milk yield in New Zealand White does

Treatments	Hormonal Levels on Day 28 of Gestation				
	T3 (ng/ml)	T4 (ng/ml)	Estradiol-17 β pg/ml	Progesterone (ng/ml)	Milk yield / doe / lactation cycle (ml)
Control	0.8 ^a \pm 0.5	21.8 ^a \pm 2.2	34.5 ^a \pm 3.8	4.2 ^a \pm 0.2	2423 ^a \pm 227
Folic Acid	0.9 ^a \pm 0.7	23.2 ^{ab} \pm 1.3	31.6 ^a \pm 2.4	6.7 ^b \pm 1.8	3761 ^b \pm 263
Iron	0.9 ^a \pm 0.4	187 ^{ab} \pm 2.2	501 ^b \pm 4.1	51 ^b \pm 0.9	3598 ^b \pm 218
Folic Acid + Iron	1.0 ^a \pm 0.6	25.2 ^b \pm 1.9	41.5 ^b \pm 4.0	6.3 ^b \pm 0.9	3743 ^b \pm 202

^a and ^b : means within the same columns with different superscripts differed ($P < 0.05$).

minimum level by a supplement of FA alone to the ration of pregnant does. TREMBLAY *et al.*, (1989) claimed that the addition of 5mg FA/kg of diet during early gestation decreased the number of dead fetuses in sows at 30 days of gestation and it also prevented both embryonic death in guineapigs (HABIZADEH *et al.*, 1986) and embryonic mortality in polytocous species (MARTINENKO, 1977). The low level of total mortality (6.2%) (Table 3) as a result of FA treatment may be explained by the rise in survival rate of fetuses during gestation (TREMBLAY *et al.*, 1989) and the improvement in litter size at birth and at weaning (40.4 and 62.5%, respectively) (Table 3).

The addition of FA to a basal diets of does increased their litter weight during all periods studied (Table 4). DUMOULIN *et al.*, (1988) found that a supplement of FA from the age of 2 to 18 weeks improved growth performance of dairy heifers. TREMBLAY *et al.*, (1989) noted that average daily live weight gain was higher for sows fed the supplement of FA than those fed the control diet. Also, growth of gilts and total litter weight from birth to 8 weeks increased linearly with FA in the diet of gestating sows (MATTE *et al.*, 1992). L'EVESQUE *et al.*, (1993) observed in white veal calves that FA improved growth ($P < 0.05$), but had no effect on feed intake. In the present study, increased litter weight in does supplemented FA during all periods studied seemed to parallel rises in litter size at birth and at weaning which coincided with a decrease in total mortality % as shown in Table 3. In addition, the increases in litter weight at birth with all treatments could be as reflection of an improvement of foetal growth.

The increase in kit weight at birth as a function of FA treatment may have been attributable to its effects on protein and nucleic acid synthesis in fetuses (TREMBLAY *et al.*, 1989). The reserve of an exceptionally high iron concentration in the liver and spleen of new-born rabbits mostly reflected on ferritin level and tissue synthesis of the fetuses (TARVYDAS *et al.*, 1968 ; UNDERWOOD, 1971).

As shown in Table 4, the increases in litter weights, at birth and 7 days of age as a function of FA + Fe treatment were less than that observed for FA or Fe treatment alone, while some complementation in that the response of kit weight at birth, 7, 14, 21 and 28 days for the combined treatment was greater than the response to either alone. These variations in litter and kit weights mostly related to the differences in litter size at birth, where litter size at birth averaged 5.6 for does supplemented with FA + Fe diet vs. 7.9 and 8.0 for FA and Fe diets, respectively (Table 3). On the

other hand, the presence and increases of both FA (MATTE and GIRARD, 1989 ; GIRARD *et al.*, 1995) and Fe molecules in solids and milk intake with the advance of age of the litter during preweaning period had a more pronounced effect on litter and kit weights.

Because of the rapidity of the hormonal level responses due to our treatments, especially FA + Fe (Table 5), it is likely that their levels may be elevated through increase the precursors or biosynthesis or release of these hormones into the blood stream. The marked increase in milk yield following FA or FA + Fe supplementation may be explained through the increase of blood and milk folates (GIRARD *et al.*, 1995) and their physiological action, since milk production was increased in sows from the first to the third week of lactation by about 30% in dams receiving FA (MATTE and GIRARD, 1989 ; NOBLET and ETIENNE, 1986). On the other hand, the increase in T4 levels as a results of these treatments (Table 5) probably reflects the physiological effects of FA and Fe on thyroid activity that related to biosynthesis of milk.

In conclusion, the supplementation of 5 mg FA or 5 mg FA plus 80 mg Fe (as FeSO₄) per kg DM above the basal levels during pregnancy will improve reproductive performance in does.

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