Effects of Dietary Supplementation of Vitamin A on Fertility and Biochemical Characteristics of Oviductal and Uterine Flushings

G. N. Egbunike, S. G. Adisa and T. O. Olaifa

Animal Physiology Laboratory, Department of Animal Science, University of Ibadan, Ibadan

Target Audience: Animal scientists; Physiologists; Nutritionists; Veterinarians.

Abstract

We decided to study the effects of dietary vitamin A supplementation on the fertility and the characteristics of the oviductal and uterine flushings of gilts because in our thermally harsh environment pigs tend to consume less feeds than needed and so expose themselves to some nutritional stress,. The experimental diets contained 12,373.8 and 16,373.8 I.U. of vitamin A per kg.

Although supplemented gilts came into puberty 21 days later, they were significantly heavier at puberty with higher conception rate than their counterparts and had a higher embryo mortality resulting in highly reduced litter size. Results on the AChE, protein and cation levels of the oviductal and uterine flushings indicate that supplementation adversely affected the quality of these tubal environments for proper embryogenesis.

The results of this study thus show that dietary vitamin A supplementation above 10,000 I.U. per kg of feed is not a commercially important factor and cannot be recommended for pig production in the humid tropics.

Key words: Vitamin A; diet; gilts; fertility; Tropics.

Introduction

Changes in the thermal environment caused by fluctuations in temperature and humidity like in the humid tropics induce a variety of physiological responses in animals (1) while vitamins have been found to affect reproduction in animals directly by acting on the reproductive organs and production of hormones or indirectly by causing unthriftiness, stunted growth, anaemia etc. In a symposium earlier on, (2) claimed that the variations existing between animals and differences in the environmental conditions on the farms coupled with unknown stress factors and animal health status, demand that a high degree of safety margin be built into the level of vitamin A added to pig diets if high levels of performance are to be achieved.

It has been known that vitamin A is necessary for the maintenance of a healthy epithelial tissue throughout the body. Premixes containing 3,000

IU of this vitamin increased fertility in pigs and reduced embryo mortality (3) while vitamin A injections improved conception rate (4,5,6) and litter size (5,6,7,8) and restored embryo survival (9) but in some studies the number of live born piglets and litter weight (10) as well as ovulation rate, embryo recovery rate and embryo diameter (11) were not affected. It is also noteworthy that even feeding of sows with vitamin A above recommended levels resulted in sows gaining more weight during pregnancy and producing more piglets with higher weight (12) unlike the noneffect of such treatment on birth weight, litter size and piglet survival (13) although injection of sows post mating did not influence total litter size, live litter size, litter weight, pig weight and number of runts or mummies (14).

With the knowledge that our environmental conditions expose our pigs to some undue stress, we decided to evaluate the effects of dietary vitamin A supplementation on the fertility and the biochemical characteristics of the oviductal and uterine flushings of porcine females.

Materials and Methods

Animals and management

Twenty one prepubertal Large White gilts five to six months of age weighing 16.5 to 47.0 kg were randomly distributed into two experimental groups equalized in age and liveweight. They were housed in two different pens in a corrugated iron-roofed concrete-floored dwarf-walled pig barn (15) and fed and provided clean water ad libitum. One group of ten gilts served as control and was fed a basal diet (Table 1) while the other was fed the basal diet supplemented with 4,000 IU of vitamin A (as Rovimix A®) per kg of feed. The gilts were tested for heat twice daily at 08.00 and 18.00 h and were weighed and bred at their first oestrus which was taken as their attainment of puberty (16).

Sample collection and analyses

All animals were weighed and slaughtered thirty five days post coitum and the reproductive tracts were separated and taken to the laboratory for further analyses. After the trimming of the adhering fatty tissues, the ovaries were weighed. The reproductive tracts were inspected for pregnancy and the uterine and oviductal flushings (UF and OF, respectively) were collected using the method of (17). These flushings were then stored frozen until analysed for acetylcholinesterase (AChE) by the method of (18), glucose by the Otoluidine colorimetric method outlined in Boehringer Diagnostic Assays Manual (19), total protein by the biuret method (20), sodium and potassium using Corning 400 photometer and calcium, zinc, magnesium, iron and copper by flaming using atomic adsorption spectrophotometry.

Table 1. Composition of basal diet.

Ingredients	Amount
Maize (yellow)	71.40
Groundnut cake	6.20
Brewer's grain	14.90
Blood meal	1.60
Bone meal	1.50
Fish meal	2.80
Oyster shell	1.00
Minovit super*	0.10
Salt (NaCl)	0.50
Total	100.00

*1 kg of Minovit super contains: Vitamin A 7.5 x 10° I.U, Vitamin D $_3$ 1.5 x 10° I.U, Vitamin B 1000 mg, vitamin B $_2$ 2,750 mg, Vitamin B $_{12}$ 5 mg, D-calcium pantothenate 5 g, Vitamin E 2.5 g, Vitamin K 1.50 g, Niacine 12.5 g, Choline chloride 60 g, ethoxyquin 5 g, MnO 16.13 g, KI 353 mg, Ferro carbonate 20.32 g.

The number of foetuses was noted and the corpora lutea were separated, counted and weighed. The crown-rump length of each foetus was then measured from the snout to the caudal end of the sacrum. Ovulation rate, conception rate, embryo mortality and litter size were determined as outlined earlier (16).

Statistical analyses

All data collected were compared using Students' t and x-square tests as outlined by Finney (21).

Results

Estimated vitamin A in the experimental diets

Using the vitamin A contents of the dietary ingredients as per (22), the levels of vitamin A in the experimental diets were 16,373.8 and 12,373.8 IU per kg of supplemented and unsupplemented diets, respectively.

Fertility

Gilts fed extra vitamin A attained puberty at 333.20 days of age 21 days later than those not supplemented. Thus age at puberty in the gilts tended to be delayed by the dietary supplementation of vitamin A even though the weight of the gilts at puberty was significantly (P<0.05) enhanced by the treatment (Table 2). Also, while ovulation rate only tended to be higher in the supplemented gilts, conception rate in this group surpassed (P<0.05) that of the unsupplemented group. While embryo mortality and crown-rump length were unaffected by the treatment, litter size at day 35 p.c. was highly significantly (P<0.01) reduced.

AChE, protein and glucose contents in UF and OF

OF is significantly (P<0.05) higher than UF in AChE but lower in glucose and protein concentration. Dietary supplementation of vitamin A significantly (P<0.05) elevated the AChE and protein contents of UF unlike glucose, which was depressed (Table 3). AChE and protein levels in OF were significantly (P<0.05) elevated and depressed respectively by dietary vitamin A supplementation while glucose level only tended to be reduced.

Table 2. Effect of dietary vitamin A supplementation on fertility in pubertal gilts (Means + SEM)

Parameters	TREATMENT	
	+4,000 IU Vit. A	+ No. Vitamin A
Age at puberty (days)	333.20 <u>+</u> 13.87	312.00 <u>±</u> 7.28
Weight at puberty (kg)	73.00 <u>+</u> 5.69	58.15 <u>+</u> 2.21*
Ovulation rate	12.00 <u>+</u> 1.76	10.00 <u>+</u> 0.82
Conception rate (%)	83.33	75.00*
Embryo mortality (%)	80.00 <u>+</u> 17.05	50.00 ± 17.57
Crown-rump length (cm)	9.27 <u>+</u> 2.93	6.8 9 ±0.27
Litter size (35days pc)	.4.00±3.00	7.25 <u>+</u> 1.31**

Treatment significantly affected values asterisked: * (P<0.05) and ** (P<0.01).

Table 3. Effect of dietary vitamin A supplementation on AChE activity, glucose and protein concentrations in uterine and oviductal flushings in pubertal gilts during the first trimester (Means ± SEM).

Parameters	TREATMENT		
	+ 4,000 IU Vit. A	+ No. Vitamin A	
AChE activity (µmole/ml/min)			
Uterine flushings	7.92 <u>+</u> 2.88	0.59 <u>+</u> 0.10*	
Oviductal flushings	31.39 <u>+</u> 8.55	2.65±0.55*	
Glucose (mg/100 ml)		- ·	
Uterine flushings	5.56±1.73	14.09±4.15*	
Oviductal flushings	0.003 ± 0.002	0.016 ± 0.009	
Protein (g/100 ml)		_	
Uterine flushings	16.15 <u>+</u> 6.75	1.71 <u>+</u> 0.46*	
Oviductal flushings	0.11 <u>+</u> 0.02	0.39 <u>+</u> 0.19*	

^{*}Treatment significantly affected values asterisked (P<0.05)

UF and OF cation lev

Sodium, potassium, calcium, magnesium, iron, copper and zinc levels in UF and OF were not significantly affected by dietary vitamin A

supplementation except in UF where sodium (P<0.05) and zinc (P<0.01) were drastically reduced (Table 4).

Table 4. Effect of dietary supplementation of vitamin A on cation concentrations in the uterine (UF) and oviductal flushings (OF) in pubertal gilts during the first trimester (Means ± Sem).

Parameters		TREATMENT	
	Flushings	+ 4,000 IU Vit. A	+ No. Vitamin A
Sodium	UF	21.48 <u>+</u> 0.18	23.23 <u>+</u> 0.67*
	OF	20.13 <u>+</u> 0.43	20.62 <u>+</u> 1.05
Potassium	UF	3.58 <u>+</u> 0.47	3.87 <u>+</u> 1.59
	OF	1.63 <u>+</u> 0.37	1.46 <u>+</u> 0.25
Calcium	UF	0.98 <u>+</u> 0.25	3.77 <u>+</u> 3.13
	OF	1.05 <u>+</u> 0.42	0.70 <u>+</u> 0.13
Magnesium	UF	0.15 <u>+</u> 0.04	0.18 <u>+</u> 0.09
	OF	0.06 <u>+</u> 0.01	0.10 <u>+</u> 0.02
Iron	UF	0.48 <u>+</u> 0.17	0.60 <u>+</u> 0.31
	OF	0.40 <u>+</u> 0.22	0.36 <u>+</u> 0.09
Copper	UF	0.06 <u>+</u> 0.03	0.11 <u>+</u> 0.07
	OF	0.05 ± 0.04	0.05 ± 0.02
Zinc	UF	0.16 <u>+</u> 0.05	0.36±0.07**
	OF	0.26 <u>+</u> 0.12	0.24 <u>+</u> 0.06

Treatment significantly affected values asterisked: * (P<0.05) and ** (P<0.01).

Discussion

The importance of vitamin A in the maintenance of the integrity of the secretory epithelial tissue cannot be denied (23). According to Hoffman-La Roche Animal Nutrition and Health Division (Basel, Switzerland) in 1991/92, growing pigs require vitamin A at 8,000 to 10,000 IU per kg feed in order to prevent nervous disorders, reproductive problems and failures as well as malformation of the newborn due to sow deficiency.

That age at puberty, ovulation rate, embryo mortality and size of the embryos (crown-rump length) were not significantly influenced by dietary vitamin A supplementation is at variance with the findings of some earlier workers

(3,4,5,6,7,8,9,24,25) but not those of others (7,10,11). Knowing, according to Toplis (26), that gilts successfully served about 20 days earlier eat less feed to produce their first litters, it may be assumed that this treatment is not a commercially important factor. This view is partly supported by the fact that in spite of the higher (P<0.05) live weight at puberty and conception rate in the supplemented animals, litter size was drastically (P<0.01) reduced. Generally, our conception rates did not deviate from those observed in temperate regions (27).

Results by (28) indicated that AChE plays a role in the regulation of the tubal microenvironment, which improves with age and

pubertal development. That OF AChE exceeded UF values is in agreement with our earlier reports (17) while the significant elevation of the AChE of the reproductive tract flushings by the dietary vitamin A treatment may suggest an excessive breakdown of acetylcholine in the membranes of embryos in the gilts.

That UF is higher than OF in protein content is in agreement with the reports of (17) and (29) while the elevation of UF proteins together with the depression of UF glucose by vitamin A supplementation may be related to the smaller litter size in this group. It is worthy to note that the litter size in these pigs are lower than the herd average as reported earlier (30) and elsewhere (31) and that the uterine secretions consist of embryotrophic factors containing uteroferrin involved in the transport of nutrients from the uterine endometrium to the conceptus (32) as well as specific uterine proteins, mainly glycoproteins, with iron-binding capacity which play a key role in embryo development (33,34,35).

As regards the cation levels of UF and OF, our results indicate generally that these fluids are stable and do not change even with dietary vitamin A supplementation. However, the drop in Na and Zn levels in the UF of animals that were supplemented with vitamin A may be construed to imply an elevation in the aldosterone secretion of the adrenal glands for the maintenance of foetal blood pressure (36).

Conclusion

There does not appear to be any evidence that dietary vitamin A supplementation is favourable to fertility and reproductive performance in gilts in the humid tropics. Hence it is not a commercially viable practice and so is not recommended.

References

- Ayo, J.O., S.B. Oladele and A. Fayomi, 1998. Stress and its adverse effects on modern swine production. Pig News and Information 19 (2): 51N-56N.
- Hardy, B., 1985. Effects of high levels of vitamin A in feeds for sows and piglets. Roche Symposium, London, UK, November, 1984. Basel, Switzerland, F. Hoffmann-La Roche & Co. AG, 14 pp.

- 3. Pochernyaeva, G.M., 1990. A vitamin premix for pigs. Zootekhniya No. 11: 42-44.
- Dun Vei, 1960. Artificial insemination of sows with semen kept at room temperature following injection of vitamin A. Anim. Breed. Abstr. 28: 2119.
- Yakimchuk, N.V., 1974. Use of vitamins to improve reproductive performance in pigs. Anim. Breed. Abstr. 44: 3344.
- Yakimchuk, N.V., 1975. The effect of vitamin A on the reproductive ability of sows. Anim. Breed. Abstr. 45: 1789.
- Briefs, S. and B.P. Chew, 1985. Effects of vitamin A and â-carotene on reproductive performance in gilts. J. Anim. Sci. 60:998-1004.
- Auldist, D.E., L. Morrish, P. Eason and R.H. King, 1998. The influence of litter size on milk production. Anim. Sci. 67: 333-337.
- Whaley, S.L., V.S. Hedgpeth and J.H. Britt,1977. Evidence that injection of vitamin A before mating may improve embryo survival in gilts fed normal or high energy diets. J. Anim. Sci. 75: 1071-1077.
- Silveira, P.R.S. Da, L.C.O.Fernandes, J.C. Moraes Filho de and W. Barioni Jr., 1998. Effect of vitamin A on the reproductive performance of sows. Revista Brasileira de Zootecnia 27: 743-748.
- Silveira, P.R.S. Da, L.C.O. Fernandes De and W. Barioni, Jr., 2000. Effect of vitamin A injection on ovulation rate, embryo development and embryo diversity in sows. Revista Brasileira de Reproducao Animal 24: 56-62.
- Kairov, V., 1998. Vitamin A requirements of sows. Svinovodstvo (Moskva) No. 6: 24-26.
- Sukhikh, N., V. Kalenyuk, N. Niyazov, V. Semenyutin and N. Sorokina, 1983. Optimum level of vitamin A in premixes for sows. Svinovodstvo, Moscow No. 4: 27.
- Pusateri, A.E., M.A. Diekman and W.L. Singleton, 1999. Failure of vitamin A to increase litter size in sows receiving injections at various stages of gestation. J. Anim. Sci. 77: 1532-1535.

- Egbunike, G.N., 1977. Sperm production rates in European boars raised and maintained in the tropics with a note on sorbitol dehydrogenase activity as an index of spermatogenesis. Niger. J. Anim. Prod. 4: 59-64.
- Egbunike, G.N., 1980. Attempts to advance puberty in swine in the humid tropics. I. Puberty and reproductive potential in gilts exposed to boars during the late growing period. Niger. Journ. Agric. Sci. 2(2): 117-120.
- Egbunike, G.N. and S.A. Adegunle, 1980. Regional variations in protein and alkaline phosphatase content in the genital tract fluid of porcine females. Zbl. Vet. Med. A. 27:319-329.
- Ellman, G.L., K.D. Courtney, V. Andres Jr. and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.
- Schmidt, F.H., 1971. Methoden der Harn- und Blutzucker-bestimung. II. Blutzucker. In: Handbuch des Diabetes mellitus (E.F. Pfeiffer, ed.), J.F. Lehmans Verlag, München, p938.
- 20. Weichselbaum, T.E., 1946. Total protein determination. Amer. J. Clin. Path. 16: 40.
- Finney, D.J., 1972. An Introduction to Statistical Science in Agriculture, 4th Ed., Blackwell Scientific Publications Ltd., Oxford.
- 22. Oyenuga, V.A., 1968. Nigeria's Feed and Feedingstuff, 3rd ed., Ibadan University Press, Ibadan.
- Gibbs, A.L., 1982. The role of vitamins in the immune response. Proc. 26th Ann. Poultry Convention, New Zealand.
- 24. Coelho, M.B. and B. Cousins, 1997. Vitamin supplementation supports higher performance. Feedstuffs 69: 10-12, 20-21.
- Paschma, J. and B. Gajda, 1999. Effects of supplemental vitamin A and â-carotene on reproductive efficiency in sows. Roczniki Naukowe Zoolechniki 26: 137-148.
- 26. Toplis, P.,1982. Feeding the breeding pig profitably. The Feed Compounder, January 1982, 6pp.

- Tomes, G.J. and H.E. Nielsen, 1982. Factors affecting reproductive efficiency of the breeding herd. In Control of Pig Reproduction (D.J.A. Cole and G.R. Foxcroft, eds.), Butterworth Scientific, London, pp 527-539.
- 27. Egbunike, G.N. and C.P. Aloamaka, 1984. Regional variations in acetylcholinesterase and phospholipids in the female porcine genital tract. In 10th Int. Congr. Anim. Reprod. & A.I., Illinois, Vol. III, Paper 481, 3pp.
- Adams, K.L., F.W. Bazer and R.M. Roberts, 1981. Progesterone-induced secretion of a retinol-binding protein in the pig uterus. J. Reprod. Fert. 62: 39-47.
- Egbunike, G.N., 1986. Response of European pigs to improved management under the humid tropical climatic conditions in Ibadan. A review. Pig News and Information 7: 315-324.
- Young, L.D., I.T. Omtvedt and R.K. Johnson, 1974. Relationship of various measures of performance with ovulation rate and number of embryos 30 days after breeding in gilts. J. Anim. Sci. 39: 480-487.
- Roberts, R.M. and F.W. Bazer, 1980. The properties, function and hormonal control of synthesis of uteroferrin, the purple protein of the pig uterus. In: Steroid Induced Uterine Proteins (M. Beato, ed.), Elsevier/North Holland Biomedical Press, Amsterdam, pp 133-149.
- 32. Chew, B.P., H. Rasmussen, M.H. Pubols and R.L. Preston, 1982. Effects of vitamin A and beta-carotene on plasma progesterone and uterine protein secretions in gilts. Theriogenology 18: 643-654.
- 33. Scherf, H. and L.J. Scott, 1989. How do vitamins affect sow productivity? Pig, January Issue, 16-17.
- 34. Christenson, R.K., F.F. Bartol, J.L.Vallet, A.A. Wiley and T.E. Spencer, 1997. Comparative study of uterine morphogenesis and protein secretion in neonatal white crossbred and Meishan gilts. Biol. Reprod. 56: 1112-1119.
- 35. Macdonald, A.A., B. Colenbrander and C.J.A.H.V. Van Vorstebosch, 1982. Physiology and endocrinology of the foetus in late gestation. In: Control of Pig Reproduction (D.J.A. Cole and G.R. Foxcroft, eds.), Butterworth Scientific, London, pp 377-404.