JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Effects of dietary vitamin supplementation and semen collection frequency on reproductive performance and semen quality in boars

I. Audet, N. Bérubé, J. L. Bailey, J.-P. Laforest and J. J. Matte

J ANIM SCI 2009, 87:1960-1970.
doi: 10.2527/jas.2008-1714 originally published online March 13, 2009

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://www.journalofanimalscience.org/content/87/6/1960

American Society of Animal Science
www.asas.org

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

Effects of dietary vitamin supplementation and semen collection frequency on reproductive performance and semen quality in boars[1]

I. Audet,* N. Bérubé,* J. L. Bailey,† J.-P. Laforest,† and J. J. Matte*[2]

*Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, 2000 College Street, PO Box 90-STN Lennoxville, Sherbrooke, Quebec, Canada, J1M 1Z3; and †Centre de Recherche en Biologie de la Reproduction (CRBR), Department of Animal Science, Laval University, Quebec, Quebec, Canada, G1V 0A6

ABSTRACT: The present study was undertaken to assess the relevance of increasing the daily provision of dietary vitamins on vitamin metabolic status and semen characteristics of boars under controlled and commercial conditions as well as to evaluate the efficiency of this vitamin supplement to allow boars to cope with intensive semen collection frequency. In the first experiment, 39 boars were allocated to 2 dietary treatments, a basal diet (control) and the basal diet supplemented with extra fat- and water-soluble vitamins (Vit). Within each treatment, boars were submitted to 2 regimens of semen collection frequency: 3 times per 2 wk (3/2) and 3 times per week (3/1) over a 12-wk period. Afterwards, all boars were intensively collected (daily) for 2 wk. A resting period of 4 wk followed, and all boars were collected 2 times per week. Thereafter, collection frequencies were reversed, and the same procedure was followed until the end of the intensive collection period. A second experiment was conducted in commercial conditions at a commercial stud, and 252 boars were randomly allocated to the control and Vit dietary treatments. All boars were collected 2 times per week over a 6-mo period. Classical measurements of ejaculate and sperm quality were assessed, and blood samples were collected throughout both experiments to quantify vitamin concentrations. In the first experiment, vitamin concentrations in blood and seminal plasma increased in Vit boars ($P < 0.05$); however, vitamin concentrations were not affected by collection frequency ($P > 0.14$). The Vit supplement did not affect sperm production or sperm quality ($P > 0.28$), although semen volume increased during the 12-wk periods for Vit boars ($P < 0.05$). The 3/1 boars produced fewer doses per ejaculate than 3/2 boars ($P < 0.01$); however, the cumulative sperm production for the 12-wk periods increased by 19% in 3/1 boars compared with 3/2 boars. In the second experiment, blood plasma concentrations of vitamin B_9 were greater ($P < 0.01$) in Vit than control boars. The vitamin supplement did not increase sperm production of boars ($P > 0.61$). In conclusion, dietary supplements of fat- and water-soluble vitamins increase the amount of vitamins available for the animal, and the collection frequencies had no effect on vitamin status. Moreover, in spite of an effect on the ejaculate volume, the dietary supplement of extra vitamins had no effect on sperm production or quality.

Key words: boar, collection frequency, semen, vitamin

©2009 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2009. 87:1960–1970
doi:10.2527/jas.2008-1714

INTRODUCTION

A major goal of commercial AI centers is to produce large quantities of semen of the greatest possible quality in an efficient period of time. Many factors influence the production and quality of semen such as breed (Kennedy and Wilkins, 1984; Ciereszko et al., 2000; Park and Yi, 2002), age (Kennedy and Wilkins, 1984; Levis, 1997), collection frequency (Swierstra and Dyck, 1976), season (McNitt and First, 1970; Swierstra, 1970; Trudeau and Sanford, 1986; Ciereszko et al., 2000; Park and Yi, 2002), and nutrition (Louis et al., 1994; Marin-Guzman et al., 1997; Audet et al., 2004). A better un-

[1]This study was supported by the Fédération des Producteurs de Porcs du Québec, Centre d'Insémination Porcine du Québec Inc., Roche Vitamins Canada Inc., and Agriculture and Agri-Food Canada. The authors are grateful to Agriculture and Agri-Food Canada staff (Sherbrooke, Québec, Canada): M. Guillette for her technical assistance; to J. Boudreau, F. Phaneuf, E. Bérubé, C. Mayrand, A. Marsh, M. Turcotte, F. Champagne, and D. Morrissette for animal care assistance; and to A. Giguère and S. Methot for advice related to statistical analyses.

[2]Corresponding author: mattej@agr.gc.ca

Received December 8, 2008.
Accepted February 26, 2009.

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

derstanding of the effects of these factors may improve the efficiency of AI centers.

In many species, dietary supplementation of extra vitamins leads to increased semen quality, quantity, or both (El-Darawany, 1999; Franchini et al., 2001; Yousef et al., 2003). In boars, a diet supplemented with selenium and vitamin E improved sperm quality (Marin-Guzman et al., 1997). Moreover, Brezinska-Slebodzinska et al. (1995) observed that extra vitamin E increased the concentration of spermatozoa in boar semen, and Audet et al. (2004) reported that extra water- and fat-soluble vitamins increased semen production during intensive semen collection.

A collection frequency of 2 to 3 times per week appears to be the most suitable frequency for boars, taking all factors into account (Frangez et al., 2005). The effect of collection frequency on semen quantity and quality, however, differs considerably among studies. It has been shown that greater collection frequencies are generally detrimental for boar semen quality (Frangez et al., 2005).

The present study was undertaken to assess the relevance of increasing the daily provision of dietary vitamins on vitamin metabolic status and semen characteristics of boars under controlled and commercial conditions as well as to evaluate the efficiency of this vitamin supplement to allow boars to cope with intensive semen collection frequency.

MATERIALS AND METHODS

All animals were used and cared for in accordance with Canadian Council on Animal Care Guidelines (1993).

Two experiments were carried out to test the hypothesis. Experiment 1 was intensive, focusing mainly on metabolic aspects related to treatments, and was conducted at the Research Centre of Lennoxville. Experiment 2 took place in commercial conditions at the Centre d'Insémination du Québec (CIPQ Inc., St-Lambert, Québec, Canada), with a large number of animals.

Exp. 1

Animals and Treatments. Fifty Duroc boars were selected at (mean ± SEM) 209.2 ± 2.79 d of age. The boars were housed individually in pens on semi-slatted floors. The average BW was 136.4 ± 1.70 kg at the initiation of treatments and 270.3 ± 2.51 kg at the end of the experiment. The boars were distributed randomly, according to their BW and age, to 1 of 2 dietary treatments: 1) basal diet (Table 1) with a vitamin premix providing concentrations corresponding to the industry average according to a survey carried out by BASF (1993), which exceeded concentrations recommended by the NRC (1998; Table 2; control, n = 25) and 2) basal diet supplemented with the control premix and extra fat-soluble and water-soluble vitamins (**Vit**; Table 2, n = 25). Premixes were kept at 4°C until the moment they were served to the animals. The daily food allowance for the whole experimental period was 3.0 kg, and the premixes were given as a top-dressing of 50 g.

Table 1. Composition of the basal diet (as-fed basis)[1]

Item[2]	Exp. 1, %	Exp. 2, %
Corn	31.4	36.5
Wheat bran	30	10
Barley	15	20
Soybean meal (48% CP)	12.2	12.5
Whole soybean	—	10
Gluten	7.5	—
Beet pulp	—	4
Alfalfa (17%)	—	2.5
Limestone	1.5	1.5
Dicalcium phosphate	0.8	1.5
Fat	0.5	—
Salt	0.5	0.5
Lysine	0.3	0.1
Methionine	0.2	0.1
Vitamins premix	—	0.7
Anti-mold[3]	0.1	—
Delquin[3] (antioxidant)	0.015	—

[1]The analytical value for riboflavin was 4.98 μg/g; for pyridoxine, 2.47 μg/g; for folates, 1.27 μg/g; and vitamin E and vitamin B_{12} were undetectable.

[2]The calculated composition for ME, CP, lysine, Ca, and P of the basal diet were (as-fed basis) 2,810 kcal/kg, 15.9, 0.96, 0.81, and 0.79%, respectively.

[3]Aliments Breton, St-Bernard, Québec, Canada.

Figure 1 provides an overview of the experimental regimen. The boars were assigned to their dietary treatment after an acclimation period of 2 wk, during which the control diet was fed. Thereafter, they received their dietary treatments until the end of the experiment. During a 3-mo period, boars were trained to mount an artificial sow for semen collection, and those that would not mount by the end of this period were culled. Therefore, 39 boars were finally kept for the experiment and distributed equally between the 2 collection frequencies within each dietary treatment. Boars were collected at an assigned frequency of 3 times per week (**3/1**, high frequency) or 3 times per 2 wk (**3/2**, regular frequency) for 12 wk. Afterwards, the animals were submitted to an intensive collection regimen (daily collection for 2 wk) to estimate the basal level of spermatogenesis, followed by a resting period of 4 wk during which the collection frequency was 2 times per week. Subsequently, there was a 12-wk period during which the collection frequencies high (3/1) and regular (3/2) were reversed with relation to the first 12 wk period and then, again, an intensive collection regimen followed (daily collection for 2 wk). Semen was collected using the gloved hand technique. The collected semen was strained through sterile gauze in a prewarmed insulated container kept at 37°C to remove gelatinous material. Semen was weighed (±1 mg), and the sperm concentration was estimated using a spectrophotometer (Novaspec II, Pharmacia Biotech, Cambridge, UK) precalibrated with a hemocytometer (Young et al., 1960). Each semen sample was diluted

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

Table 2. Daily vitamins provided by the premix in each treatment (control = basal diet for mineral and vitamins, and Vit = control supplemented with fat-soluble vitamins and water-soluble vitamins)[1,2]

Vitamin	Exp. 1 control[1]	Exp. 2 control[2]	Exp. 1 and 2 Vit[1]
Vitamin A, KIU	20	37.5	100
Vitamin D, KIU	3	4.5	10
Vitamin E, IU	120	168.75	600
Menadione, mg	2	8.4	10
Choline, mg	400	2,812.5	4,000
Pantothenic acid, mg	40	45	400
Riboflavin, mg	10	15	100
Folic acid, mg	4	15	40
Niacin, mg	50	93.75	500
Thiamine, mg	2	3.75	20
Pyridoxine, mg	6	7.5	60
Vitamin B_{12}, mg	0.04	0.075	0.4
Biotin, mg	0.5	2.5	5

[1]Fifty g of premix were given as top-dressing. Analytical values (means ± SEM) for vitamin E were 687.7 ± 8.5 IU for Vit vs. 116.6 ± 3.6 IU for control; for riboflavin, 96.6 ± 3.5 mg for Vit vs. 10.4 ± 0.25 mg for control; for pyridoxine, 66.2 ± 1.8 mg for Vit vs. 6.4 ± 0.22 mg for control; for folic acid, 35.9 ± 0.94 mg for Vit vs. 4.9 ± 0.29 mg for control; and for vitamin B_{12}, 0.39 ± 0.01 mg for Vit vs. 0.04 ± 0.004 mg for control. Each premix provided the following daily: Mn as manganous oxide, 60 mg; Zn as zinc oxide, 200 mg, Fe as ferrous sulfate, 200 mg; Cu as copper sulfate, 30 mg; I as calcium iodate, 0.54 mg; and Se as selenite, 0.54 mg.

[2]Premix for control for Exp. 2 provided the following daily: Mn as manganous oxide, 150 mg; Zn as zinc oxide, 375 mg, Fe as ferrous sulfate, 375 mg; Cu as copper sulfate, 93.75 mg; I as calcium iodate, 2.25 mg; Se as selenite, 0.375 mg; and Co, 0.375 mg.

to a final concentration of 3×10^9 spermatozoa per dose of 85 mL in a commercial extender (BTS extender; Bio'dil, Genes Diffusion, Douai, France).

Sperm morphology was determined on slides stained with eosin-nigrosin containing glucose and TES-Tris (Bamba, 1988) during the 12-wk periods (3 times) and during the intensive collection periods (3 times). Morphology was assessed using a light microscope (400×) by counting 100 sperm in 2 areas of 1 slide per ejaculate. They were classified as normal sperm, abnormal tails, abnormal heads, loose heads, proximal cytoplasmic droplets, and distal cytoplasmic droplets. Sperm motility and progressive motility were performed with a Hamilton Thorn Motility Analyzer, HTM 2000 (Hamilton Thorn Research, Danvers, MA) and was determined on each ejaculate during the 12-wk periods and at 1, 4, 5, 10, and 14 d of intensive collection periods. The following settings were used: frames acquired, 10; frame rate, 30/s min; contrast, 1 min; size, 2 min; lo/hi size gates, 0.6 to 2.6; lo/hi intensity gates, 0.5 to 2.5; nonmotile head size, 9; nonmotile intensity, 125; medium path velocity value, 20 μm/s; low path velocity value, 5 μm/s; slow cells motile, yes; threshold straightness, 45; temperature, 37.5°C; chamber, microcell 20 μm; phase contrast.

Sampling. Blood samples were taken from a jugular vein by venipuncture throughout the experiment (Figure 1) to measure concentrations of the fat- (vitamin E) and water-soluble (B_2, B_6, B_9, and B_{12}) vitamins included in the dietary supplements. Samples were collected in EDTA-containing tubes (10 mL; Becton Dickinson and Co., Rutherford, NJ), centrifuged for 12 min at 4°C at 1,800 × *g*, and the plasma was aliquoted and frozen at −20°C for subsequent vitamin analyses. Samples were taken at the beginning of the experiment (before allocation to treatments), before each regular collection period, and before and after each intensive collection period. Seminal plasma and sperm samples were taken at the same time as blood samples except for the first sample because the boars were not yet trained for semen collection. A portion of the undiluted ejaculate was centrifuged for 20 min at 4°C and 1,800 × *g*. The seminal plasma was aliquoted and frozen at −20°C for vitamin analyses, and the sperm pellet obtained from the centrifugation of 2 mL of semen was resuspended in 500 μL of H_2O and frozen at −20°C for vitamin E analyses.

Vitamin Measurements. Concentrations of vitamins B_2, B_6, B_9, and B_{12} were determined in blood plasma and seminal plasma, whereas vitamin E concentrations were measured in blood plasma and sperm pellets. All vitamins were measured according to Audet et al. (2004). Vitamin E (α-tocopherol) measurements were validated for the sperm pellets. Instead of 250 μL of blood plasma, 15 μL of 5% BHT were added to the frozen sperm pellet of 2 mL of semen. The validation tests for the measurement of vitamin E in sperm showed intra- and interassay CV of 5.4 and 7.6%, respectively.

Exp. 2

This experiment was done in commercial conditions at CIPQ Inc. (St-Lambert, Québec, Canada). A total of 252 Duroc boars (young and mature) were selected for the experiment; 39 of them were culled mainly because of poor semen quality not attributed to treatments. Boars were distributed randomly, according to their

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

age and sperm production (mature boars), to 1 of the 2 dietary treatments: 1) basal diet (Table 1) with a vitamin premix used by CIPQ Inc. (control, n = 110), and 2) basal diet supplemented with the control premix and Vit corresponding to Exp. 1 (Table 2, n = 103). Of the 213 boars in Exp. 2, 117 were pubertal (8 mo) and 96 were mature (15 to 20 mo). Boars were balanced within dietary treatments along the time of the year. The daily

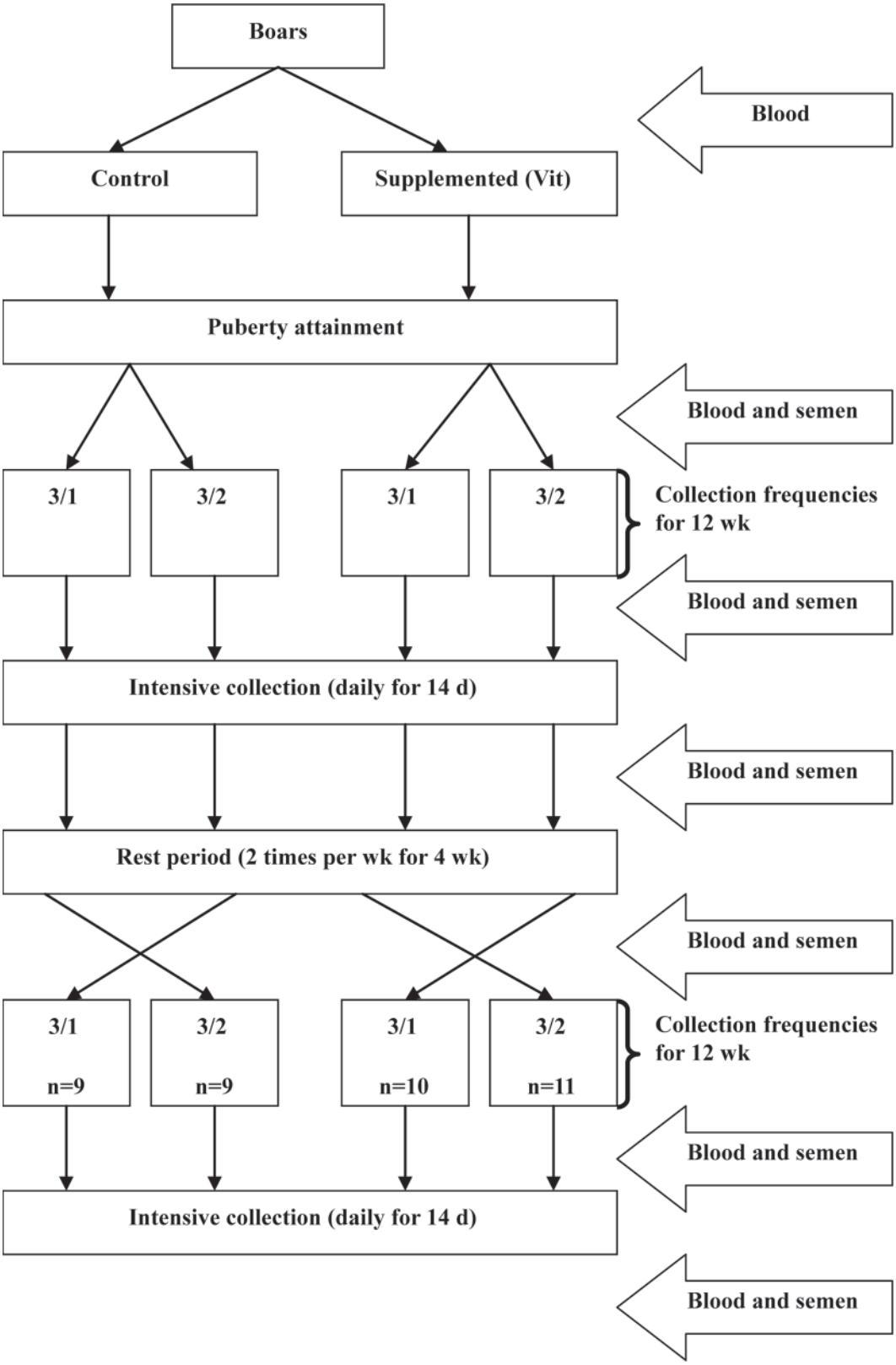

Figure 1. Outline of experimental design used for Exp. 1. Control = basal diet for mineral and vitamins, and Vit = control supplemented with water- and fat-soluble vitamins.

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

Table 3. Concentrations of vitamins in blood plasma of boars during the different periods of Exp. 1 according to dietary treatments[1,2]

Period	Dietary treatment[3]	B_2, pg/mL	B_6, ng/mL	B_9, ng/mL	B_{12}, pg/mL	E, μg/mL
12-wk period	Control	135 ± 3.8	237 ± 6	40.7 ± 1.74	350 ± 14	1.70 ± 0.06
	Vit	157 ± 3.8	273 ± 7	52.5 ± 1.52	512 ± 15	2.57 ± 0.08
Intensive collection period	Control	134 ± 3.8	239 ± 5	40.7 ± 1.72	326 ± 13	1.72 ± 0.06
	Vit	156 ± 7.5	270 ± 5	53.0 ± 1.39	477 ± 14	2.60 ± 0.08
4-wk rest period	Control	136 ± 7.5 NS[4]	255 ± 8	39.5 ± 2.39	343 ± 19	1.71 ± 0.07
	Vit	157 ± 7.5 NS	287 ± 8	56.8 ± 2.26	497 ± 22	2.53 ± 0.08

[1]Values are means ± SEM of the values taken at the beginning and the end of the period.
[2]Diet effect ($P < 0.05$) except for B_2 during the rest period.
[3]Control = basal diet for mineral and vitamins, and Vit = control supplemented with fat-soluble vitamins and water-soluble vitamins.
[4]NS = nonsignificant.

food allowance for the whole experimental period was 3.0 kg, and the premixes were given as a top-dressing of 50 g. Boars were collected 2 times per week during 6 mo. Sperm concentration, volume, and total number of sperm per ejaculate were recorded as well as the subjective motility classed as 1 to 5, where 5 represented no sperm motility. Three blood samples were collected from 60 boars (15 young-control, 15 mature-control, 15 young-Vit, and 15 mature-Vit) during the experiment (before treatment, at 3 mo, and at the end of the experiment). Blood sampling and preparation of plasma were the same as Exp. 1. Vitamins B_{12} and folates were measured according to Audet et al. (2004).

Statistical Analyses

For Exp. 1, the data were analyzed using the MIXED procedure (SAS Inst., Cary, NC; Littell et al., 1996) according to a 2 × 2 factorial arrangement of treatments with dietary supplements (control and Vit) and semen collection frequencies (3/1 and 3/2) as main effects. The analysis was conducted separately for each period of the experiment: the 12-wk periods, the intensive collection periods, and the rest period.

Vitamins assayed in blood and seminal plasma were analyzed as repeated measurements (means at the initiation and final of each period), and polynomial contrasts were used to evaluate the time effect. Seminal plasma vitamins were expressed as concentration of vitamins per milliliter of seminal plasma. Differences were considered significant at $P < 0.05$. The boar was considered as the experimental unit. The following model was used: $Y_{ij} = \mu + B_i + F_j + (B_i \times F_j) + e_{ij}$, where Y_{ij} = dependent variable, B_i = vitamin supplements, F_j = collection frequency, $B_i \times F_j$ = interaction between vitamins supplements and collection frequency, and e_{ij} = residual error.

Sperm production and quality were also analyzed as repeated measurements. For sperm production during the 12-wk periods, measurements were pooled in 3 means, each representing an average value for total sperm, concentration, and volume for 1 mo; during the intensive collection periods and the 4-wk rest period, each ejaculate was taken into account in the repeated analysis (respectively, 14 and 7 ejaculates). Sperm morphology was evaluated at the beginning, the middle, and the end of the 12-wk and intensive collection periods. The repeated analysis for sperm motility during the 12-wk periods was done as for sperm production (3 means, 1 per month), whereas during the intensive collection periods, motility was evaluated on d 1, 4, 7, 10, and 14; no sperm morphology or motility measurements were done during the 4-wk rest period.

The data from Exp. 2 were analyzed in the same manner as for Exp. 1 except that it was according to a 2 × 2 factorial arrangement of treatments with dietary supplements (control and Vit) and age of boar (young and mature) as main effects, and only 1 period was taken into account (the 6-mo period). The following model was used: $Y_{ij} = F_j + (B_i \times F_j) + e_{ij}$, where Y_{ij} = dependent variable, F_j = age of boars, B_i = vitamin supplements, $B_i \times F_j$ = interaction between vitamin supplements and age of boars, and e_{ij} = residual error.

RESULTS

Exp. 1

Blood Vitamin Status. Blood plasma concentrations of vitamins B_2, B_6, B_9, B_{12}, and E were greater ($P < 0.05$) in Vit than in control boars for all periods of the experiment except for the vitamin B_2 in the rest period ($P = 0.17$; Table 3). Blood plasma concentrations of vitamins were not affected ($P > 0.26$) by collection frequencies (data not shown). During the intensive collection periods, whatever the treatments, blood plasma concentrations of vitamins B_2, B_{12}, and E decreased ($P < 0.03$), whereas those of vitamin B_6 increased ($P < 0.03$). During the rest period, concentrations of vitamins B_6 and B_{12} increased ($P < 0.05$), whereas concentrations of vitamin E further decreased ($P < 0.01$).

Seminal Plasma and Sperm Vitamin Status. Seminal concentrations of vitamins B_2, B_9, and

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

Table 4. Concentrations of vitamins B_2, B_6, B_9, and B_{12} in seminal plasma and vitamin E in sperm of boars during the different periods of Exp. 1[1,2]

Period	Dietary treatment[3]	B_2, pg/mL	B_6, ng/mL	B_9, ng/mL	B_{12}, pg/mL	E, μg/mL
12-wk periods	Control	39.5 ± 2.6	273 ± 9 NS[4]	1.86 ± 0.09	4,440 ± 203	0.126 ± 0.010
	Vit	46.7 ± 2.3	282 ± 11 NS	2.53 ± 0.13	5,671 ± 250	0.183 ± 0.015
Intensive collection periods	Control	30.9 ± 1.9	234 ± 10 NS	1.49 ± 0.07	3,750 ± 214	0.097 ± 0.010
	Vit	38 ± 1.9	243 ± 11 NS	2.04 ± 0.1	5,061 ± 203	0.140 ± 0.016
4-wk rest period	Control	28.6 ± 2.3 NS	229 ± 12 NS	1.35 ± 0.09	4,024 ± 306	0.084 ± 0.012
	Vit	32.4 ± 1.9 NS	240 ± 14 NS	1.92 ± 0.15	5,090 ± 261	0.127 ± 0.019

[1]Values are means ± SEM of the values taken at the beginning and the end of the period.
[2]Diet effect ($P < 0.05$) except for B_2 during the rest period and B_6 throughout the experiment.
[3]Control = basal diet for mineral and vitamins, and Vit = control supplemented with fat-soluble vitamins and water-soluble vitamins.
[4]NS = nonsignificant.

B_{12} were greater in Vit boars than in control boars for all periods of the experiment ($P < 0.04$), except for the seminal concentrations of vitamin B_2 in the rest period ($P = 0.12$; Table 4). The seminal concentration of vitamin B_6 was not affected by dietary treatment ($P > 0.29$). Seminal concentrations of vitamins B_6 and B_9 decreased during the 12-wk periods for the boars collected 3/1 from 289.2 ± 15.1 to 255.1 ± 15.8 ng/mL and from 2.43 ± 0.22 to 1.81 ± 0.13 ng/mL for B_6 and B_9, respectively, whereas they were stable for 3/2 boars (283.0 ± 9.6 and 2.30 ± 0.11 ng/mL, respectively, for B_6 and B_9; interaction frequency of collection × time, $P < 0.01$). During the intensive collection periods, concentrations of all vitamins decreased ($P < 0.01$). Moreover, during the intensive collection periods, there was a residual effect of the previous collection frequency, the concentration of seminal B_9 was greater for boars collected 3/2 than boars collected 3/1 (1.94 ± 0.1 vs. 1.62 ± 0.08 ng/mL, respectively; interaction frequency of collection × time, $P < 0.03$). During the rest period, no effect of time ($P > 0.08$) was observed on the concentrations of any vitamin except for vitamin B_9, which increased ($P < 0.01$) from 1.39 ± 0.09 to 1.92 ± 0.15 ng/mL.

In sperm, vitamin E concentration was greater for Vit than control boars for all periods of the experiment ($P < 0.06$; Table 4). The concentration of vitamin E in the sperm increased from 0.139 ± 0.011 to 0.170 ± 0.015 μg/mL during the 12-wk periods and from 0.055 ± 0.007 to 0.156 ± 0.016 μg/mL during the rest period, but decreased during the intensive collection periods from 0.170 ± 0.015 to 0.0644 ± 0.0075 μg/mL ($P < 0.01$). During the 12-wk periods, boars collected 3/2 showed an increase of vitamin E in semen from 0.148 ± 0.017 to 0.197 ± 0.023 μg/mL, whereas the semen concentration of vitamin E in sperm was maintained for boars collected 3/1 (0.136 ± 0.011 μg/mL; $P < 0.05$). During the intensive collection periods, boars that were collected 3/1 had decreased concentrations of vitamin E in semen compared with boars collected 3/2 (0.098 ± 0.012 and 0.138 ± 0.016 μg/mL, respectively; $P < 0.04$).

Sperm Quality. During the 12-wk periods, no diet or collection frequency effects were observed on the percentages of sperm motility, progressive motility, or morphology ($P > 0.51$; Table 5). The overall means ± SEM were 89.5 ± 0.54% of motile sperm, 84.8 ± 0.27% of sperm with progressive motility, and 83.9 ± 0.85% of sperm with normal morphology. During the intensive collection periods, no diet or residual effects of the collection frequency were observed on quality criteria ($P > 0.18$; Table 5). However, both motility and progressive motility decreased during the intensive collection periods ($P < 0.01$; Figure 2). Nevertheless, no effect of time was observed for sperm morphology ($P > 0.26$).

Sperm Production. There was no diet effect in any period ($P > 0.30$) for the total number of sperm per ejaculate and sperm concentration (Table 5). Overall means ± SEM were $4.38 \times 10^{10} \pm 0.09 \times 10^{10}$ sperm per ejaculate and $3.11 \times 10^{8} \pm 0.07 \times 10^{8}$ sperm per mL. The increase of semen volume for Vit boars during the 12-wk periods (145.1 ± 7.1 mL to 162.9 ± 7 mL) was greater than for the control boars (142.8 ± 7.2 mL to 151.2 ± 6.8 mL; interaction diet × time, $P < 0.05$).

There was a greater sperm production for 3/2 than 3/1 boars during the 12-wk periods ($5.14 \times 10^{10} \pm 0.13$ and $3.59 \times 10^{10} \pm 0.08$ sperm per ejaculate, respectively; $P < 0.05$). However, the cumulative sperm production per boar during the 12-wk periods was greater for 3/1 than 3/2 boars ($1.291 \times 10^{12} \pm 0.05 \times 10^{12}$ and $1.108 \times 10^{12} \pm 0.04$ total sperm, respectively; $P < 0.01$).

During the intensive collection periods, sperm production decreased from d 1 to 7 and remained stable thereafter. A residual effect of collection frequency was observed during the first 7 d (interaction collection frequency × time, $P < 0.05$; Figure 3); i.e., the initial decrease in sperm production during the first 4 d for 3/2 boars was greater than for 3/1 boars.

All criteria (sperm production, semen concentration, and volume) increased during the rest period ($P < 0.01$). In controls, sperm concentration was greater for boars collected 3/2 than 3/1 ($3.479 \times 10^{8} \pm 0.16$ and $3.009 \times 10^{8} \pm 0.11$ sperm per mL, respectively), whereas it was the opposite for Vit boars ($2.598 \times 10^{8} \pm 0.09$ and $3.343 \times 10^{8} \pm 0.13$ sperm per mL, respectively, for 3/2 and 3/1 boars; interaction diet × collection frequency, $P < 0.01$).

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Accqs 001AAU9974 on May 29, 2014

Table 5. Sperm quality and sperm production according to the age of boars and the periods of Exp. 1

Period	Dietary treatment[1]	Collection frequency treatments	Volume, mL[2,3]	Sperm concentration, $\times 10^8$ spz/mL[2,4]	Total sperm production, $\times 10^{10}$ spz/ejaculate[2,5]	Motility,[6] %	Progressive motility,[6] %	Normal sperm,[7] %
12-wk periods	Control	3/1	137.6 ± 5.0	2.756 ± 0.107	3.633 ± 0.126	89.9 ± 0.71	84.9 ± 0.49	84.7 ± 1.56
		3/2	153.8 ± 6.2	3.593 ± 0.162	5.136 ± 0.192	90.0 ± 1.11	84.9 ± 0.51	84.4 ± 1.84
	Vit	3/1	144.5 ± 6.3	2.711 ± 0.115	3.552 ± 0.094	90.1 ± 1.20	85.1 ± 0.55	84.4 ± 1.75
		3/2	162.7 ± 5.2	3.370 ± 0.145	5.142 ± 0.173	88.0 ± 1.18	84.4 ± 0.56	82.3 ± 1.66
Intensive collection periods	Control	3/1	129.0 ± 2.8	1.742 ± 0.048	2.189 ± 0.072	89.0 ± 0.53	84.0 ± 0.55	84.6 ± 1.56
		3/2	125.4 ± 2.7	1.855 ± 0.059	2.279 ± 0.085	88.6 ± 0.64	83.9 ± 0.63	85.0 ± 1.78
	Vit	3/1	136.8 ± 2.7	1.624 ± 0.044	2.128 ± 0.061	87.7 ± 0.67	82.8 ± 0.66	82.0 ± 1.73
		3/2	133.8 ± 2.6	1.825 ± 0.059	2.342 ± 0.081	87.8 ± 0.53	83.1 ± 0.57	81.4 ± 1.88
4-wk rest period[8]	Control	3/1	152.9 ± 5.6	3.009 ± 0.110	4.554 ± 0.221	—	—	—
		3/2	157.1 ± 6.0	3.479 ± 0.164	5.094 ± 0.190	—	—	—
	Vit	3/1	158.5 ± 5.7	3.343 ± 0.130	5.004 ± 0.176	—	—	—
		3/2	189.0 ± 5.9	2.598 ± 0.089	4.752 ± 0.153	—	—	—

[1]Control = basal diet for mineral and vitamins, and Vit = control supplemented with fat-soluble vitamins and water-soluble vitamins.

[2]For sperm production during the 12-wk periods, measurements were pooled in 3 means each representing an average value for total sperm (spz = spermatazoa), concentration, and volume for 1 mo; during the intensive collection periods and the 4-wk rest period, each ejaculate was taken into account in the repeated analysis (respectively, 14 and 7 ejaculates).

[3]Interaction diet × time $P < 0.05$ for the 12-wk periods.

[4]Interaction diet × collection frequency $P < 0.01$ for the 4-wk rest period.

[5]Effect of collection frequency $P < 0.05$ for the 12-wk periods.

[6]The repeated analysis for sperm motility and progressive motility during the 12-wk periods was done as for sperm production (3 means), whereas during the intensive collection periods, these criteria were evaluated on d 1, 4, 7, 10, and 14.

[7]Sperm morphology was evaluated at the beginning, the middle, and the end of the 12 wk and intensive collection periods.

[8]No sperm morphology or motility measurements were done during the 4-wk rest period.

Exp. 2

In commercial conditions, blood plasma concentrations of vitamin B_9 were greater ($P < 0.01$) in Vit than in control boars and particularly for mature boars (interaction diet × age × time, $P < 0.03$; Table 6). The blood plasma concentration of vitamin B_{12} was not affected ($P > 0.48$) by dietary treatment (Table 6). There was an age effect ($P < 0.05$) but no dietary treatment effect for total sperm production, sperm concentration, volume, and quality of motility score ($P > 0.61$; Table 7). No interaction between dietary treatment and season was observed for any criteria measured ($P > 0.31$).

DISCUSSION

Vitamin Status

Vitamins increased in blood plasma (B_2, B_6, B_9, B_{12}, and E) of boars supplemented with extra vitamins, regardless of the collection frequency. Moreover, most of the water-soluble vitamins measured in blood plasma were apparently transferred to seminal plasma, except for vitamin B_6. In previous experiments, it was also observed that blood plasma concentrations of vitamin B_9 (Wallock et al., 2001; Audet et al., 2004) and vitamin B_{12} (Boxmeer et al., 2007) were highly correlated with the concentrations of seminal B_9 and B_{12}, respectively. However, in contrast to the present results, blood concentrations of vitamins B_2 in the study of Audet et al. (2004) were not correlated to the seminal concentrations. This discrepancy might be related to an interaction with the genotype because 3 breeds of boars (Yorkshire, Landrace, and Duroc) were used by Audet et al. (2004). In the present experiment, a direct transfer of vitamin E to spermatozoa was observed in accordance with the report of Marin-Guzman et al. (1997); vitamin E was measured in spermatozoa because of its absence in seminal plasma (Marin-Guzman et al., 1997; Audet et al., 2004).

Seminal concentrations of vitamin B_6 and B_9 and sperm concentrations of vitamin E were less for boars collected 3/1 than 3/2 in the 12-wk periods. Moreover, during the intensive semen collection, all vitamins measured in seminal plasma and sperm decreased. It seems that boars would transfer more vitamins toward sperm as the frequency of ejaculation increases. Nevertheless, the difference between 3/1 and 3/2 boars is likely to be insufficient to affect the overall vitamin requirement of the animal, as indicated by the lack of systemic effect on concentration of those vitamins in the blood.

On a daily basis, taking into account the daily excretion of the vitamins in seminal plasma between the boars collected 3/1 and 3/2, it can be estimated that the daily excretion of the vitamins was 2 times greater for boars collected 3/1 than for boars collected 3/2 (117.9 vs. 63.2 ng/d for folates; 273.8 vs. 162.6 ng/d for B_{12}; 2,066.5 vs. 1,288.0 pg/d for B_2; and 15.4 vs. 8.8 μg/d for B_6). However, blood plasma concentrations of vitamins were not affected by collection frequencies. This can be attributable to the fact that the amount of vitamins B_2, B_6, and folates excreted daily in seminal plasma represents less than 0.3% of the total amount in blood plasma; for vitamin B_{12} the corresponding value was 3%. The quantities of vitamins in seminal plasma are, therefore, negligible compared with the quantity of vitamins in blood plasma and are unlikely to affect homeostasis and the daily requirement of these vitamins.

Nevertheless, the case of vitamin B_{12} appears peculiar in seminal plasma because the ratio to blood plasma, as shown above, is approximately 10 times greater than for the other vitamins. In humans, it was shown that transcobalamin and haptocorrin (cobalamin binding proteins) are present in normal seminal plasma in concentrations that are 10- to 20-fold greater than in

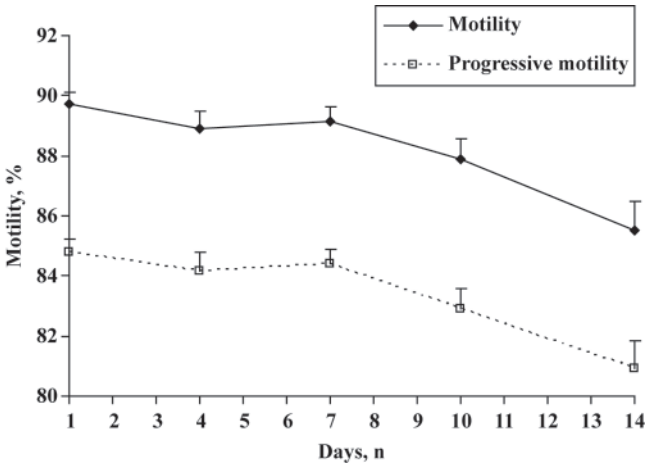

Figure 2. Changes in the sperm quality during the intensive collection periods. Overall mean ± SEM for percentage of motile sperm and percentage of sperm with progressive motility were 88.2 ± 0.3 and 83.4 ± 0.3%, respectively (time effect, $P < 0.0006$).

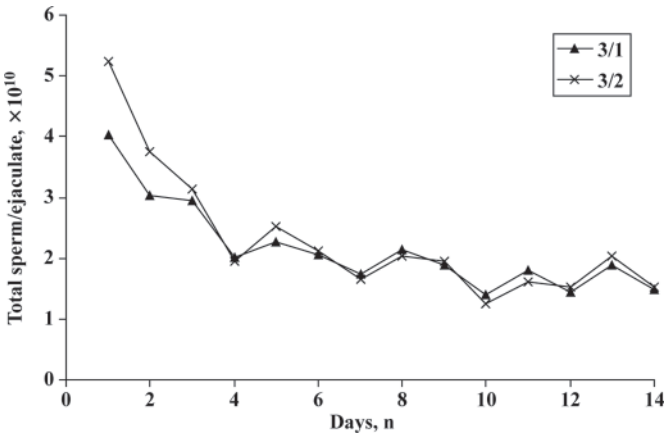

Figure 3. Changes in the total sperm number produced during the intensive collection periods, according to collection frequency treatments and days of collection. Semen collection frequencies of 3/1 and 3/2 refer to boars collected 3 times per 1 wk and 3 times per 2 wk, respectively, during the 12-wk periods. Overall mean ± SEM was 2.24 × 10^{10} ± 0.04 × 10^{10} total sperm number. Interaction collection frequency × time $P < 0.05$ for the first 7 d of the intensive collection periods.

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

Table 6. Concentrations of vitamins in blood plasma according to dietary treatments and age of boars in commercial conditions in Exp. 2

Age of boars	Dietary treatment[1]	Samples	B_{12},[2] pg/mL	B_9,[3] ng/mL
Young	Control	1	414.5 ± 49.0	54.2 ± 5.2
		2	465.8 ± 47.5	50.4 ± 5.1
		3	506.8 ± 48.1	54.1 ± 5.1
	Vit	1	415.4 ± 49.0	56.5 ± 5.2
		2	468.1 ± 47.5	55.2 ± 5.1
		3	485.1 ± 48.2	61.9 ± 5.1
Mature	Control	1	433.4 ± 52.4	57.1 ± 5.7
		2	395.6 ± 52.4	46.9 ± 5.8
		3	404.1 ± 53.6	51.7 ± 5.9
	Vit	1	406.3 ± 54.5	48.6 ± 6.0
		2	459.2 ± 54.5	65.6 ± 6.0
		3	446.5 ± 55.7	70.5 ± 6.1

[1]Control = basal diet for mineral and vitamins, and Vit = control supplemented with fat-soluble vitamins and water-soluble vitamins.
[2]Vitamin B_{12} was not affected ($P > 0.48$) by the dietary treatment.
[3]Interaction diet × age × time ($P < 0.03$) for vitamin B_9.

blood (Fukuda and Yamada, 1992; Hansen and Nexo, 1992). Although the presence of these cobalamin binding proteins has not been evaluated in boars, the present accumulation of vitamin B_{12} in seminal plasma indicates a functional binding mechanism in this metabolic pool. Vitamin B_{12} acts as a coenzyme in several biochemical reactions (McDowell, 2000); although its role is not well defined, there is some evidence that it affects sperm variables (Watson, 1962). In fact, vitamin B_{12} deficiency during gestation and lactation affects future male reproductive systems and inhibits the maturation of spermatogenic cells in rats (Watanabe et al., 2003, 2007). Vitamin B_{12} is therefore an essential constituent for spermatogenesis, and the present dietary provision in control boars was likely sufficient to meet the metabolic needs for reproducing boars.

Sperm Quality

It is generally recognized that boars subjected to a high semen-collection frequency have poor semen quality (Meding, 1975; Swierstra and Dyck, 1976; Cameron, 1985; Schilling and Vengust, 1987; Strzezek et al., 1995). In the present study, no effects on motility or morphology of sperm were observed between the 3/1 and 3/2 boars during the 12-wk periods. However, during the intensive semen collection periods, when boars were collected daily during 14 d, the motility decreased, but the morphology was not affected. It appears that a chronic increase of collection frequency (3/1 vs. 3/2) was not stressful enough to affect semen quality as it was the case with an acute increase of collection frequency (daily during 14 d).

Although an increase of vitamins was observed in seminal plasma of boars supplemented with high level of vitamins, sperm quality was not influenced by dietary treatments, regardless of the collection frequency. Others (Brezinska-Slebodzinska et al., 1995, in boars; Geva et al., 1996, in humans; Hsu et al., 1998, in rats; and Surai et al., 1998, in cockerels) reported that a supplementation with vitamins E, C, or both reduced the production of reactive oxygen species and improved semen quality. In the present experimental conditions, it seems that concentrations of vitamin E in the control diet were sufficient to maximize sperm quality.

Sperm Production

Collection frequency affected total sperm collected. High semen collection frequency decreased sperm pro-

Table 7. Sperm quality and sperm production according to dietary treatments and age of boars in commercial conditions in Exp. 2

Age of boars	Dietary treatment[1]	Motility score	Volume,[2] mL	Sperm concentration, × 10^8 spz[3]/mL	Total sperm production, × 10^{10} spz/ejaculate[2]
Young	Control	2.78 ± 0.01	154.9 ± 1.3	3.827 ± 0.028	5.587 ± 0.042
	Vit	2.75 ± 0.01	156.8 ± 1.3	3.891 ± 0.033	5.733 ± 0.044
Mature	Control	2.72 ± 0.01	188.1 ± 1.7	3.487 ± 0.029	6.228 ± 0.055
	Vit	2.80 ± 0.01	184.6 ± 1.6	3.614 ± 0.033	6.270 ± 0.055

[1]Control = basal diet for mineral and vitamins, and Vit = control supplemented with fat-soluble vitamins and water-soluble vitamins.
[2]Effect of age, $P < 0.05$.
[3]spz = spermatazoa.

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

duction per ejaculate, but increased cumulative sperm collected over a period of time. This response was consistent with the results of Strzezek et al. (1995).

Supplements of extra vitamins did not influence sperm production no matter the collection frequency. We previously reported (Audet et al., 2004) that dietary supplements of water-soluble vitamins increased semen production during intensive semen collection. Again, as for vitamin status, it cannot be ruled out that the difference with the present experiment can be related to an interaction with genotypes, because 3 different breeds of boars were used by Audet et al. (2004). Nevertheless, taking into account the number of animals involved (controlled and commercial conditions) and the factorial arrangement of treatments with collection frequencies, the present study can be regarded as more reliable in terms of vitamin homeostasis and its impact on the reproductive performance of boars.

In conclusion, results of the present study indicate that dietary vitamin supplements affect blood and seminal pools of vitamins. Most of the vitamins seem to be transferred from the blood to the seminal plasma. However, in spite of this metabolic transfer, there were no marked treatment effects on sperm production or semen quality. Dietary supplements of water- and fat-soluble vitamins do not appear to be efficient to allow the boar to cope with semen frequencies.

LITERATURE CITED

Audet, I., J. P. Laforest, G. P. Martineau, and J. J. Matte. 2004. Effect of vitamin supplements on some aspects of performance, vitamin status, and semen quality in boars. J. Anim. Sci. 82:626–633.

Bamba, K. 1988. Evaluation of acrosomal integrity of boar spermatozoa by bright field microscopy using an eosin-nigrosin stain. Theriogenology 29:1245–1251.

Boxmeer, J. C., M. Smit, R. F. Weber, J. Lindemans, J. C. Romijn, M. J. Eijkemans, N. S. Macklon, and R. P. Steegers-Theunissen. 2007. Seminal plasma cobalamin significantly correlates with sperm concentration in men undergoing IVF or ICSI procedures. J. Androl. 28:521–527.

Brezinska-Slebodzinska, E., A. B. Slebodzinski, B. Pietras, and G. Wieczorek. 1995. Antioxydant effect of vitamin E and glutathione on lipid peroxydation in boar semen plasma. Biol. Trace Elem. Res. 47:69–74.

Cameron, R. D. 1985. Factors influencing semen characteristics in boars. Aust. Vet. J. 62:293–297.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals, Vol. 1. CCAC, Ottawa, Ontario, Canada.

Ciereszko, A., J. S. Ottobre, and J. Glogowski. 2000. Effects of season and breed on sperm acrosin activity and semen quality of boars. Anim. Reprod. Sci. 64:89–96.

BASF. 1993. Vitamin Supplementation Rates for U.S. Commercial Poultry, Swine and Dairy Cattle. BASF Corp., Mount Olive, NJ.

El-Darawany, A. A. 1999. Improving semen quality of heat stressed rams in Egypt. Indian J. Anim. Sci. 69:1020–1023.

Franchini, A., M. L. Bergonzoni, C. Melotti, and G. Minelli. 2001. The effects of dietary supplementation with high doses of vitamin E and C on the quality traits of chicken semen. Arch. Geflügelk. 65:76–81.

Frangez, R., T. Gider, and M. Kosec. 2005. Frequency of boar ejaculate collection and its influence on semen quality, pregnancy rate and litter size. Acta Vet. (Brno) 74:265–273.

Fukuda, M., and S. Yamada. 1992. Serum B_{12} proteins versus seminal plasma binder. J. Nutr. Sci. Vitaminol. (Tokyo) 114–117.

Geva, E., B. Bartoov, N. Zabludovsky, J. B. Lessing, L. Lerner-Geva, and A. Amit. 1996. The effect of antioxydant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program. Fertil. Steril. 66:430–434.

Hansen, M., and E. Nexo. 1992. Cobalamin binding proteins in human seminal plasma. Scand. J. Clin. Lab. Invest. 52:647–652.

Hsu, P. C., M. Y. Liu, C. C. Hsu, L. Y. Chen, and Y. L. Guo. 1998. Effects of vitamin E or C or both on reactive oxygen species-related lead toxicity in the rat sperm. Toxicology 128:169–179.

Kennedy, B. W., and J. N. Wilkins. 1984. Boar, breed and environment factors influence semen characteristics of boars used in artificial insemination. Can. J. Anim. Sci. 64:833–843.

LevisD. G. 1997. Managing postpubertal boars for optimum fertility. Compendium's Food Anim.: Med. Manage. 19:S17–S23.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Inst. Inc., Cary, NC.

Louis, G. F., A. J. Lewis, W. C. Weldon, P. M. Ermer, P. S. Miller, R. J. Kittok, and W. W. Stroup. 1994. The effect of energy and protein intake on boar libido, semen characteristics, and plasma hormone concentrations. J. Anim. Sci. 72:2051–2060.

Marin-Guzman, J., D. C. Mahan, Y. K. Chung, J. L. Pate, and W. F. Pope. 1997. Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality, and subsequent fertilization rates in mature gilts. J. Anim. Sci. 75:2994–3003.

McDowell, L. R. 2000.Vitamins in Animal and Human Nutrition. Iowa State Univ. Press, Ames, IA.

McNitt, J. I., and N. L. First. 1970. Effects of 72-hour heat stress on semen quality in boars. Int. J. Biometeorol. 14:373–380.

Meding, J. H. 1975. Influence of semen collection on breeding efficiency of a.i. boars. Nord. Vet. Med. 27:153–160.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. Natl. Acad. Press, Washington, DC.

Park, C. S., and Y. J. Yi. 2002. Comparison of semen characteristics, sperm freezability and testosterone concentration between Duroc and Yorkshire boars during seasons. Anim. Reprod. Sci. 73:53–61.

Schilling, E., and M. Vengust. 1987. Frequency of semen collection in boars and quality of ejaculates as evaluated by the osmotic resistance of acrosomal membranes. Anim. Reprod. Sci. 12:283–290.

Strzezek, J., W. Kordan, J. Glogowski, P. Wysocki, and D. Holody. 1995. Influence of semen-collection frequency on sperm quality in boars, with special reference to biochemical markers. Reprod. Domest. Anim. 30:77–84.

Surai, P., I. Kostjuk, G. Wishart, A. Macpherson, B. Speake, R. Noble, I. Ionov, and E. Kutz. 1998. Effect of vitamin E and selenium supplementation of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes and liver. Biol. Trace Elem. Res. 64:119–132.

Swierstra, E. E. 1970. The effect of low ambient temperature on sperm production, epididymal sperm reserves, and semen characteristics of boars. Biol. Reprod. 2:23–28.

Swierstra, E. E., and G. W. Dyck. 1976. Influence of the boar and ejaculation frequency on pregnancy rate and embryonic survival in swine. J. Anim. Sci. 42:455–460.

Trudeau, V., and L. M. Sanford. 1986. Effect of season and social environment on testis size and semen quality of the adult Landrace boar. J. Anim. Sci. 63:1211–1219.

Wallock, L. M., T. Tamura, C. A. Mayr, K. E. Johnston, B. N. Ames, and R. A. Jacob. 2001. Low seminal plasma folate concentrations are associated with low sperm density and count in male smokers and non-smokers. Fertil. Steril. 75:252–259.

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

Watanabe, T., S. Ebara, S. Kimura, K. Maeda, Y. Wanatabe, H. Wanatabe, S. Kasai, and Y. Nakano. 2007. Maternal vitamin B_{12} deficiency affects spermatogenesis at the embryonic and immature stages in rats. Congenit. Anom. (Kyoto) 47:9–15.

Watanabe, T., K. Ohkawa, S. Kasai, S. Ebara, Y. Nakano, and Y. Watanabe. 2003. The effects of dietary vitamin B_{12} deficiency on sperm maturation in developing and growing male rats. Congenital Anomalies 43:57–64.

Watson, A. A. 1962. Seminal vitamin B_{12} and sterility. Lancet 29:644.

Young, D. C., R. H. Foote, A. R. Turkheimer, and H. D. Hafs. 1960. A photoelectric method for estimating the concentration of sperm in boar semen. J. Anim. Sci. 19:20–25.

Yousef, M. I., G. A. Abdallah, and K. I. Kamel. 2003. Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim. Reprod. Sci. 76:99–111.

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014

References This article cites 31 articles, 7 of which you can access for free at:
http://www.journalofanimalscience.org/content/87/6/1960#BIBL

Citations This article has been cited by 1 HighWire-hosted articles:
http://www.journalofanimalscience.org/content/87/6/1960#otherarticles

Downloaded from www.journalofanimalscience.org at RM Cooper Library/Acqs 001AAU9974 on May 29, 2014