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RESEARCH ARTICLE

Ovsynch Synchronisation Programme Combined with Vitamins and Minerals in Underfed Cows: Biochemical, Hormonal and Reproductive Traits [1] [2]

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Summary

The aim of present study was to investigate the effects of Ovsynch synchronisation programme combined with vitamins and minerals upon biochemical, hormonal and reproductive parameters for improving post partum (pp) reproductive performance of underfed dairy cows. Thirty-six healthy cows, aged 3-10 years-old, were used with a minimum 43 days pp. Following the first blood sampling (as baseline), 25 days before synchronisation (GnRH injection), the body condition scores (BCSs, 1-5 scale) were determined. After balancing the BCSs (2.56, mean), animals were then divided into four trial groups, as control (Group I, n=7), vitamin ADE (II, n=10), mineral mix (III, n=9) and combination group (IV, n=10). Overall, the results suggest that; i) Ovsynch synchronisation programme, supplemented with vitamins and minerals, favourably affected the plasma levels of vitamin A only, ii) desirable effects of supplements upon the presence of luteal structure and ultimate pregnancy rate were evident in vitamin group only, iii) glucose (tendency) as well as some protein and lipid levels were progressively changed by negative energy balance (NEB), and iv) the low initial cyclic activity and poor BCS seemed to diminish the overall success rate of synchronisation programme used in underfed dairy cows early pp.

Keywords: *Cow, Synchronisation, Vitamin, Mineral, Metabolism, Reproduction, NEB*

Yetersiz Beslenen İneklerde Vitamin ve Mineral Uygulamalarıyla Kombine Edilen Ovsynch Senkronizasyon Programı: Biyokimyasal, Hormonal ve Reprodüktif Özellikler

Özet

Sunulan çalışmada, yetersiz bakım-besleme koşulları altındaki sütçü ineklerde vitamin ve mineral uygulamalarıyla kombine edilen Ovsynch senkronizasyon programının biyokimyasal, hormonal ve reprodüktif parametreler üzerine etkilerinin postpartum (pp) reprodüktif performansın artırılmasındaki rolü araştırıldı. Yaşları 3-10 arasında değişen, doğumunun üzerinden en az 43 gün geçmiş, 36 baş sütçü inek kullanıldı. İlk kan örneklerinin (bazal seviye) alınmasından sonra, senkronizasyon (GnRH enjeksiyonu) öncesi 25. günde, vücut kondisyon skorları (VKS, 1-5 skalası) belirlendi. Ardından, hayvanlar VKS yönünden dengelendikten (ort. 2,56) sonra, kontrol (Grup I, n=7), vitamin ADE (II, n=10), mineral mix (III, n=9) ve kombine grup (IV, n=10) olmak üzere dört deney grubuna ayrıldı. Sonuç olarak, erken pp dönemindeki sütçü ineklerde; i) Ovsynch senkronizasyon programıyla birlikte kullanılan vitamin ve minerallerin, sadece plazma vitamin A düzeylerini olumlu yönde etkilediği, ii) anılan katkının, luteal yapı varlığı ve gebelik oranı üzerine istenilen etkilerinin sadece vitamin grubunda belli olduğu, iii) glikoz (nispeten), protein ve lipid düzeylerinin, çalışma boyunca negatif enerji balansına (NEB) bağlı olarak değiştiği, ve iv) başlangıçtaki düşük siklik aktivite ve zayıf VKS düzeylerinin, senkronizasyon programının genel başarı oranını azaltabildiği kanısına varıldı.

Anahtar sözcükler: *İnek, Senkronizasyon, Vitamin, Mineral, Metabolizma, Reprodüksiyon, NEB*

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INTRODUCTION

In herd reproductive management, the aim for optimal production is generally to obtain a live calf each year. But, generally a prolonged calving-interval (around 30 days) is seen [1]. Hence, the pp period is very critical to minimize any delay. Undoubtedly, this requires an accurate detection of oestrus, expected to result in high conception rates. However, as appreciated, all these are possible only under optimal conditions [2].

Considering the literature on fertility, one may conclude that the main reason of infertility is poor management, *i.e.* nutrition, poor oestrus detection and incorrect timing of AI [1]. Obviously, in dairy industry, the demands for production of more milk from genetically high-yielding dairy cows have inevitably resulted in functional reproductive disorders [2]. In harsh climate/long-lasting winter conditions (about 2.000 m altitude), reproductive disorders are quite common [3-5], due to poor management [3,6], influencing all the productive traits concerned [7].

To overcome reproductive problems, numerous synchronisation techniques have been used [8,9]. Progesteron (P_4) [10], $PGF_{2\alpha}$ [11] and GnRH plus $PGF_{2\alpha}$ based protocols [8] have frequently been used either alone or in combination [12]. Of these, the Ovsynch protocol, as developed by Pursley *et al.*[13], could be used with considerable success (33-77% pregnancy) [14-17], but superior results were mostly seen in normally cycling cows [2]. Therefore, this method is generally used for controlled-AI programmes in dairy cows in the US [18]. It was further recommended that, since this protocol presently is in use across the world, key improvements could be made easily by its modification strategies in future. Also, the GnRH/hCG administrations following the AI improved the pregnancy/calving rates [11,19]. Of the latter, GnRH could be used after AI to prevent possible embryonic mortality in suspected herds [1,2].

Apart from the synchronisation, the nutrition itself has profound effects on reproduction, especially for those individuals with poor condition [2,20-22]. Few data are available concerning the effects of minerals and vitamins on oestrous behaviour [23]. But, their deficiencies have apparent effects on reproduction [1,2,24]. Furthermore, supplementation with vitamins [8,25-27] and minerals [28,29] improved the pregnancy rate. Vitamin A plays a crucial role in the synthesis of steroid hormones, such as P_4 [30]. Since dietary β-carotene (precursor of vitamin A) prevents oxidative stress and plays a crucial role in immunostimulation [31], its deficiency decreases the reproductive outcome by impairing the ovarian function and uterine environment. Indeed, it was recently observed that vitamin A+ D+ E and vitamin E plus selenium prevented inevitable lipid peroxidation and oxidative stress due to long-distance transportation [32]. Likewise, in ram-lambs, compensating feeding with the urea-molasses-mineral block meal improved, to some extent, the levels of libido, plasma leptin and LH [33].

Therefore, the aim of this study was to investigate the effect of vitamins and minerals combined with Ovsynch synchronisation programme upon the biochemical, hormonal and reproductive parameters in underfed dairy cows pp.

MATERIAL and METHODS

Animals

In this study, 36 cows (35 Holstein and 1 Swedish Red) aged 3-10 years-old (mostly 6-8 years for all groups) were used in a private dairy farm (in Erzurum) during March-August, 2009. Cows (> 42 days pp) were assumed healthy; without abnormal vaginal discharge and having normal ovaries/uterus.

Management

For feeding, the cows were given daily a diet in varying contents (firstly, grass hay + silage + concentrate meal; secondly, grass hay + concentrate meal; and finally, whole forage meal of barley and trefoil), amounts (5-10 kg forage/silage, 2 kg concentrate and barley-trefoil *ad libitum*) and durations (from one week to 3-4 months). They were supplied water *ad libitum*.

Animals were housed freely in indoor shelters with sufficient bedding and air conditioning together with semi-free access to outdoor pens especially during daytime.

Body Condition Scoring and Milk Yields

The BCSs were determined by 1-5 scale (1-emaciated; 5-obese) using the method of Çolak and Uçar [22]. Daily milk yields were calculated from the computer-based surveillance data.

Experimental Groups

After balancing the *BCSs* (mean: 2.56±0.03 units, $P>0.05$), animals were divided into four trial groups, as control (Group I, n=7), vitamin (Group II, n=10), mineral (Group III, n=9) and combination group (Group IV, n=10) *(Table 1)*.

Vitamin-Mineral Administrations

Fourteen days prior to the synchronisation, the injections of *placebo*, vitamin (Ademin®, CEVA-DIF, Istanbul-TR; 10 cc, IM), mineral (Minerasol®, INTERHAS, Ankara, as manufactured by Richter Pharma Ag Wels- Austria; 5 cc, in 10 cc warm physiological serum, IM deeply), and vitamin plus mineral (vit. - min.) injections were made in Group I, II, III and IV, respectively. In Group IV, both sides of the neck were used for injections, as one side each.

Table 1. *Plasma vitamin, mineral and BCS levels in underfed dairy cows subjected to oestrus synchronisation (Ovsynch) combined with vitamins and/or minerals*

Tablo 1. *Yetersiz beslenen sütçü ineklerde vitamin ve/veya minerallerle kombine edilen östrus senkronizasyonu (Ovsynch) sonrası plazma vitamin, mineral ve VKS düzeyleri*

Parameter	Groups											
	Control (n=7)			Vit.* (n=10)			Min.** (n=9)			Vit. + Min. (n=10)		
	BL	Day 22	Day 46	BL	Day 22	Day 46	BL	Day 22	Day 46	BL	Day 22	Day 46
VitA (ng/µl)	0.16±0.06	0.32±0.05	0.51±0.14	0.13±0.02^{a}	0.47±0.09^{ab}	0.65±0.13^{b}	0.14±0.04	0.21±0.05	0.49±0.1	0.22±0.06	0.23±0.06	0.4±0.10
VitD (ng/ml)	68.6±10.2	66±15.9	46.1±9.7	54.3±6.7	76.3±13.4	57.1±11.7	65.9±3.5	91.1±15.8	42.6±4.7	62.1±3.9	97.3±28.2	57.2±6.8
VitE (ng/µl)	9.3±1.4	13.2±2.6	12.5±2.4	10.1±1.1	10.8±2.5	15.2±2.2	9.6±1.9	10.2±1.7	13.4±2.2	8±1.4	8±1.9	12±2.4
Fe (µg/dl)	80.1±6.3	71.9±3.9	70.3±7.7	72.2±4.4	67.6±4.6	90.2±3.3	74.3±6.5	69±5.2	82.9±6	73.9±3.2	73.2±4.5	86±5.2
Na (mmol/l)	134.7±0.6	134.3±1.3	130.4±0.9	134.5±0.9	133±0.9	130.1±0.9	133.2±1.1	133±1.1	131.4±0.3	135.6±1.1	133±0.9	132.7±0.5
K (mmol/dl)	25.8±0.4	23±0.7	21.9±1.3	26.4±0.3	25.5±0.4	22.5±1.0	26.4±0.2	25.5±0.4	23.2±1.2	26.3±0.6	26.2±0.8	22.2±0.8
P (mg/dl)	4.6±0.3	3.5±0.2	4±0.7	4.3±0.2	3.6±0.2	4±0.4	5.3±0.3	3.6±0.3	4.4±0.4	4.7±0.2	3.3±0.2	4.1±0.4
Mg (mg/dl)	0.2±0	0.31±0.03	0.37±0.04	0.22±0.01	0.24±0.02	0.3±0.03	0.21±0.01	0.24±0.02	0.33±0.04	0.21±0.01	0.22±0.01	0.34±0.02
Zn (µg/dl)	31.3±5.7^{a}	43.3±2.9^{a}	92.7±10.8^{b}	38±4.8^{a}	45±3.1^{a}	97±7.6^{b}	36.6±2.9^{a}	51.8±5.6^{a}	99.6±10.3^{b}	44.3±3.5^{a}	58.9±8.4^{a}	97.8±5.9^{b}
BCS	2.5±0.03	2.55±0.05	2.38±0.03	2.57±0.05	2.55±0.06	2.42±0.04	2.56±0.07	2.56±0.06	2.48±0.06	2.61±0.07	2.56±0.07	2.43±0.05
Milk, l/day	7.79±1.78			9.54			7.24±2.18			10.06±1.75		

The values (mean ± SEM) having different superscripts within the same raw for each group are significantly different from each other (P<0.05)
BL: Baseline (11 days before vit. - min. injection), Day 22 : 22 days after vit. -min. injection, Day 46 : 46 days after vit. -min. injection
** **Vitamins:** 500.000 IU vit. A, 75.000 IU vit. D_3 and 50 mg vit. E per ml*
*** **Minerals:** 65 mg calcium phosphinate, 282 mg potassium chloride, 300 mg calcium gluconate, 50 mg potassium iodide, 50 mg sodium iodide, 19.64 mg magnesium chloride, 12.96 mg iron chloride, 0.164 mg cobalt chloride and 0.1355 mg zinc chloride per ml*

Ovarian Cyclicity, Oestrus, Oestrus Synchronisation and AIs

At the beginning, the ovarian cyclicity (hyperactivity) was detected by pedometry [23]. Signs of oestrus (the presence of mounting/standing, vaginal mucous, Graaf follicle and/or absence of active CL) were recorded [2]. For synchronisation, the first GnRH, Lecirelin acetate (75 mg, Dalmarelin®, VETAŞ, İstanbul; IM) was injected on the first day. On the 8th day of the injection, a $PGF_{2\alpha}$, Dinoprost tromethamine- THAM salt (25 mg, Enzaprost®, CEVA-DİF; IM) was given. The first intrauterine artificial inseminations (AIs) were performed (n=36) using frozen semen (0.50 cc straws, 10x10^{6} total sperm/dose, >50% motility; EGEVET, İzmir) between the 20th-26th h after the second GnRH injection on day 10. Twelve days after the first AI, 25 mg injection of the third GnRH was given. The second (n=35) and third (n=15) AIs were also performed, as appropriate, on the 21st and 42nd days after the first AI, respectively.

Blood Sampling

Totally, three blood samples were collected routinely by venipuncture into the vacutainer tubes containing EDTA (10 ml, Becton Dickinson Co., USA), as follows:

The first samples were collected 25 days before the first GnRH injection (11 days before the vitamin and mineral injections). The second sampling was made just before the injection of $PGF_{2\alpha}$ on the 8th day of the GnRH injection. The third samplings were made on the 21st day of the second AI. In other words, the first sampling (11 days before vit.-min. injection) was assigned as baseline (BL), while the second and third samples were collected on the 22nd and 46th days of vit. -min. injection, respectively.

Once collected, the samples of plasma were obtained by centrifugation at 3.000 *g* for 15 min at 4°C and stored at -20°C until the analyses.

Laboratory (Biochemical and Hormonal) Analyses

The biochemical analyses of vitamins by HPLC (HP-Agilent 1100), Zn mineral by atomic absorption spectrophotometry (Perkin-Elmer A Analyst 800) and others (Fe, Na, K, P, Mg) as well as metabolic parameters by automated spectrophotometry (Cobas 6000, Roche) were made according to their specific methods, as directed by the manufacturer guidelines.

For determination of P_4 levels, plasma samples in 96-well microtitre plates were directly analysed by using a double-antibody EIA (Enzyme immunoassay) technique, as described by Prakash et al.[34]. The analyses were carried out in Ondokuzmayıs University, Faculty of Veterinary Science, Division of Physiology (Samsun). The coverage of microtitration plate was made by using the method of Güven et al.[35]. Standard series used for measurement of P_4 were prepared as; 0 (zero), 0.25, 0.50, 1.0, 2.0, 4.0, 8.0 and 16.0 ng/ml. For quality control, a single reference (control) sample was used for each of low and high concentrations. The intensity of colour was measured at 450 nm with an 8-channel microtitration plate spectrophotometer (DAS, Digital and Analogue Systems, A3, Italy). The results obtained in duplicates were calculated by using the 4 parameters logistic curve-fit. Intra- and inter-assay

coefficients of variations for the controls of low and high concentrations were 4% and 22% *vs.* 12% and 13%, respectively.

Rectal Palpation

Examinations were performed routinely by two separate trained people before, during and after the study for detecting reproductive tract disorders, the presence of Graaf follicle, CL or pregnancy (*e.g.* fremitus, asymmetry, foetal findings). The presence of follicle/CL was monitored by 21 days intervals following each of three AIs. Finally, the ultimate pregnancy rate was determined routinely at the end of study, 108 days following the first AI.

Statistical Analyses

Data from the concentrations of vitamins and minerals as well as hormonal and metabolic parameters of the experimental groups against time (4 groups x 3 times) were analysed by pair-wise one-way ANOVA [36] with Tukey's multiple comparison post hoc test using SigmaStat (Version 2.03, SPSS Inc., USA). Chi-square test was also used, as appropriate. The values were given as mean ± SEM. Differences were considered significant when $P<0.05$.

RESULTS

The rates of initial regular cycles, with no significant difference ($P>0.05$) between the groups (I-IV), were given in *Fig. 1a*.

Vitamin and mineral levels as well as the BCS and milk values were shown in *Table 1*. It should be noted that, following deep IM injections of minerals, no apparent side effects were seen, except for minor local temporary oedema in three animals. Regarding the sampling times, the comparisons of variations were presented as differences within the groups.

Considering the vitamin levels, vitamin A tended to

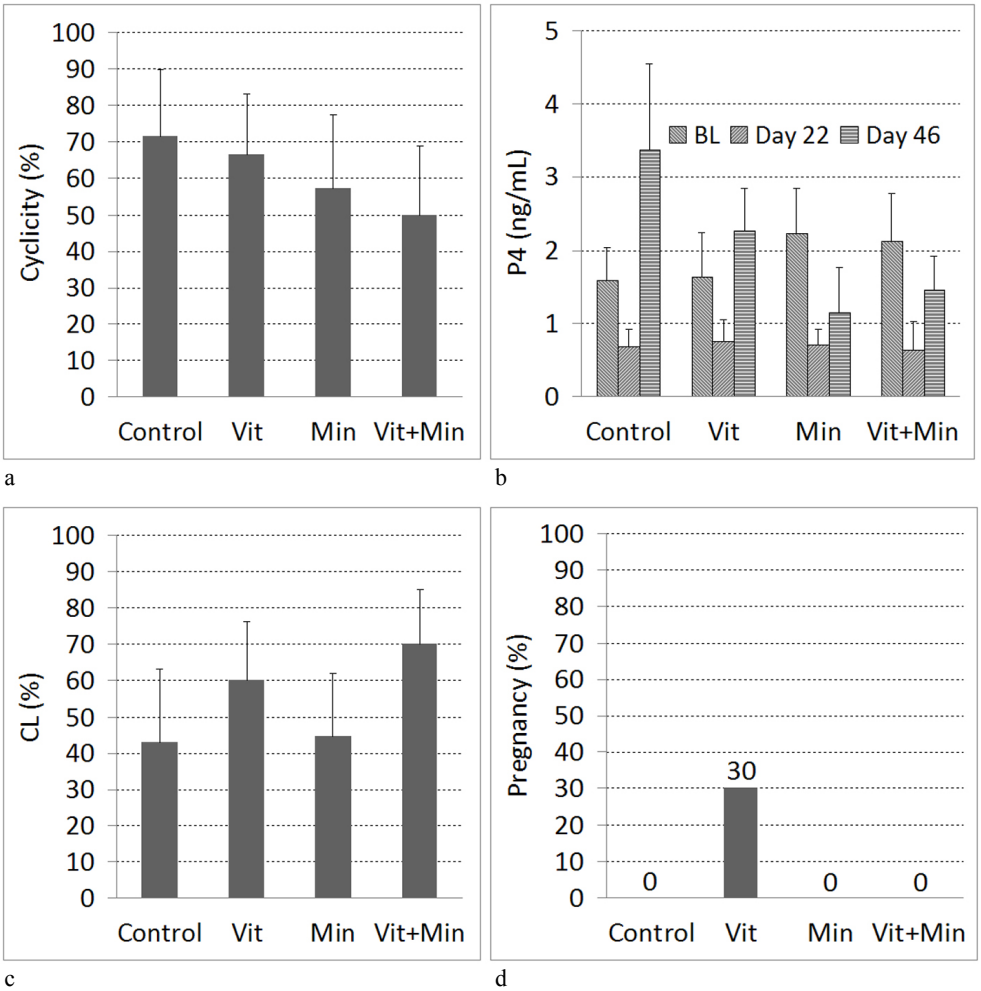

Fig 1 (a-d): *Initial cyclicity (cyclic activity) rates (a), progesterone levels (b), presence of luteal structure, CL (c) and pregnancy rates (d) in experimental groups*
a- *Cyclic activity of Day BL,* ***b-*** *P_4 levels,* ***c-*** *CL of Day_{AI} 42,* ***d-*** *Pregnancy of Day_{AI} 108*
BL: Baseline (11 days before vit. - min. injection), Day 22 : 22 days after vit. - min. injection, Day 46 : 46 days after vit. - min. injection. Day AI: Day after the 1st AI

Şekil 1 (a-d): *Deney gruplarındaki başlangıç siklisitesi (siklik aktivite) oranları (a), progesteron düzeyleri (b), luteal yapı varlığı (c) ve gebelik oranları (d)*
a- *Basal düzey günü siklik aktivite,* ***b-*** *P_4 düzeyleri,* ***c-*** *Suni tohumlama sonrası 42. günde CL,* ***d-*** *Suni tohumlama sonrası 108 günlük gebelik*

increase during the course of study, however a significant increase was observed on the third blood sampling in Group II only (P<0.05). For vitamin D, there was a slight decrease by the time in controls; however the levels of the second sampling were numerically higher than the others within (II-IV). For vitamin E, the changes were negligible.

For minerals, the levels of Fe, Na, K, P and Mg remained virtually unchanged in the second samples. But, Zn levels were significantly higher on the third sampling in all groups, regardless of treatment.

The BCS levels remained virtually unchanged by the second samplings. But, then, there were similar numerical declines *(Table 1)*. Also, milk yield was similar (low) between groups.

The initial levels of P_4 in all groups declined considerably by the second sampling (just prior to $PGF_{2\alpha}$ injection) *(Fig. 1b)*. But, the levels of third samplings then increased considerably in Group I and II, or slightly in Group III and IV until the 21st day of the second AI.

The levels of active CL following the 21st day of each AI were not significantly different from each other *(Table 2)*. Nevertheless, the cows in Group IV had a considerably high rate of CL following the second AIs *(Fig. 1c)*, but it declined afterwards. However, 3 cows in Group II sustained pregnancy, by the end of study, significantly different from others *(Fig. 1d)*.

The mean levels of metabolic parameters were given in *Table 3*. For glucose, there were slight decreases by the

Table 2*. Presence of luteal structure following the artificial insemination of postpartum dairy cows*
Tablo 2. *Postpartum sütçü ineklerde suni tohumlama sonrası luteal yapı varlığı*

Days (after the AIs)	Groups (I-IV)			
	Control (n=7)	Vit. (n=10)	Min. (n=9)	Vit.+Min. (n=10)
	CL, %	CL, %	CL, %	CL, %
21	0	10 (1/10)	0	0
42	42.86 (3/7)	60 (6/10)	44.44 (4/9)	70 (7/10)
63	28.57 (2/7)	50 (5/10)	22.22 (2/9)	10 (1/10)
108	0	30 (3/10)*	0	0

** Pregnancy (P<0.05)*

Table 3. *Plasma biochemical parameters of underfed dairy cows subjected to oestrus synchronisation (Ovsynch) combined with vitamins and/or minerals*
Tablo 3. *Yetersiz beslenen sütçü ineklerde vitamin ve/veya minerallerle kombine edilen östrus senkronizasyonu (Ovsynch) sonrası plazma biyokimyasal parametreleri*

Parameter	Groups											
	Control (n=7)			Vit. (n=10)			Min. (n=9)			Vit.+Min. (n=10)		
	BL	Day 22	Day 46	BL	Day 22	Day 46	BL	Day 22	Day 46	BL	Day 22	Day 46
GLU (mg/dl)	56.3±0.7	47.6±2.1	44.8±3.9	57.9±1.9	46.9±3	47.7±3.4	57.2±2.1	44.7±3.9	46.2±3.6	57.3±1.2	45.7±2.5	47.9±2.5
TCOL (mg/dl)	133.7±6.5	113.4±8.6	121.8±7.5	135.7±7.8	115.3±7.8	117.2±8.2	142.3±9.5	118.9±9.0	127±6.3	118.1±7.1	97.3±5.8	115±8.3
HDL (mg/dl)	107.7±4.7	101.4±6.3	106.2±6.3	111.9±5.3	100.5±7	101.2±6.5	111.9±7.9	105.4±7.7	109.6±5.5	98.3±5.8	89.2±5.5	100.2±6.8
LDL (mg/dl)	26.3±3	17.9±2.8	20.8±2.7	25.3±2.6	18.8±1.7	18.5±2.4	31.7±2.5	21.7±2.5	21.9±1.6	18.7±1.8	13.4±1.5	17.4±1.9
VLDL (mg/dl)	2.9±0.1	3.4±0.4	5.3±0.4	3±0.3	2.6±0.4	4.4±0.2	$2.7±0.2^{ab}$	$2.3±0.4^{a}$	$5.8±1.0^{b}$	$2.8±0.2^{a}$	$2.6±0.4^{a}$	$6.3±0.8^{b}$
TG (U/l)	14±0.8	16.6±1.8	27±2.4	15.2±1.2	13.1±1.8	22.0±0.9	$13.9±0.7^{ab}$	$11.7±1.9^{a}$	$28.4±5.0^{b}$	$14.2±1.3^{ab}$	$12.2±2.1^{a}$	$31.1±4.0^{b}$
T.Prot. (mg/dl)	8.1±0.2	8.1±0.2	8.5±0.2	8.3±0.2	8.4±0.2	8.4±0.3	8.1±0.2	8.1±0.2	8.3±0.2	7.9±0.2	8.2±0.2	8.5±0.2
Albumin (g/dl)	3.1±0.1	3.1±0.1	3.1±0.1	3.3±0.1	3.1±0.1	3.2±0.1	3±0.1	3.1±0.1	3.1±0	3.3±0.1	3.3±0.1	3.4±0.1
Globulin (g/dl)	5±0.2	5.1±0.2	5.4±0.3	5.1±0.2	5.2±0.3	5.2±0.4	5±0.2	5±0.2	5.2±0.2	4.6±0.2	4.9±0.2	5.1±0.1
AST (U/l)	78.3±2.1	98.3±6.2	97.5±4.1	72.2±2.7	91.1±3.7	90.4±3.8	76.4±4.5	94.8±5.1	98.4±7.3	74.3±2.4	87.9±3.8	87.3±2.6
ALT (U/l)	35.3±1.2	39.7±1.9	31.7±3.3	33.9±2.1	36±1.6	29±2.4	33.8±2.5	39.1±2.1	31.1±2.4	31.3±1.5	33.4±1.9	26±2.6
LDH (U/l)	1196±37	1204±47	1218±43	1128±48	1182±54	1162±52	1114±53	1137±28	1159±52	1087±31	1081±38	1103±42
GGT (U/l)	18.1±0.4	14.9±0.6	15±0.8	19.6±1.2	16.1±1.3	17.1±2.8	17.6±1.1	15.6±1.4	15.1±1.5	15.7±1	13.1±0.8	13.3±0.7
T. Bil. (mg/dl)	0.09±0.01	0.09±0.01	0.17±0.03	0.08±0.01	0.08±0.02	0.13±0.02	0.09±0.01	0.12±0.02	0.13±0.02	0.09±0.01	0.1±0	0.17±0.02
AMYL (U/l)	17.1±1.9	19.6±1.6	16.8±1.8	18.7±1.5	20.8±1.8	21.2±1.8	19.7±1.7	19.6±1.8	22.4±2.1	18.8±1.7	19.7±1.8	23.2±1.9
CRE (mg/dl)	$0.91±0.05^{b}$	$0.84±0.05^{ab}$	$0.67±0.03^{a}$	$0.94±0.02^{b}$	$0.97±0.05^{b}$	$0.77±0.03^{a}$	$0.9±0.05^{b}$	$0.89±0.05^{b}$	$0.7±0.03^{a}$	0.98±0.04	0.91±0.04	0.83±0.03
UA (mg/dl)	0.47±0.04	0.41±0.04	0.33±0.02	0.39±0.04	0.35±0.02	0.36±0.03	0.44±0.02	0.36±0.02	0.4±0.04	0.44±0.04	0.37±0.03	0.37±0.04

The values (mean ± SEM) having different superscripts within the same raw for each group are significantly different from each other (P<0.05)
BL: Baseline (11 days before vit. - min. injection), Day 22 : 22 days after vit. - min. injection, Day 46 : 46 days after vit. - min. injection

second and third samplings as compared to BL levels in all groups. Likewise, for lipids, the levels of TCOL, HDL and LDL tended to decrease, except for Group IV on the third samplings. By contrast, the levels of VLDL and TG increased significantly in Groups III and IV on the third samplings, but with slight increases in others. For proteins, the levels of T. protein, albumin and globulin remained unchanged. For liver enzymes; the levels of ALT, LDH and GGT remained unchanged, but the AST levels tended to increase on the second and third samplings, as compared to BL levels for all the groups. The levels of T. bilirubin remained within the reference limits. There were slight changes for the levels of amylase. Finally, the initial levels of creatinine remained virtually unchanged, but then, followed by significant decrease, except for those in Group IV with a slight decline only.

DISCUSSION

The initial rates of regular oestrus cycles of cows varied from 50% (Group IV) to 71.4% (Group I). Such a considerably high proportion (up to 50%) of non-cyclic animals could have been presumably related to poor BCS (around 2.5 units) because of underfeeding [20,22,37,38] and poor management [2,9]. Indeed, for example, there were missing pedometer in some cows, poor feeding history (forage and concentrate meal), and undesirable air flow within the barn, etc.

The cows in Group IV had a considerably high rate (70%) of CL following the second AI, as compared to those rates (from 42.9% to 60%) in other groups. No oestrus signs were observed before the third AI. Furthermore, especially after vitamin (alone or with mineral) injections, the ovaries palpated were predominantly in larger-size in Group IV, as compared to those in Group I and III, particularly. But, only a 30% of cows in Group II sustained embryogenesis. This may partially be explained by considerably high initial cyclic activity rates (66.7%) in that group, thus allowing for superior results. Although there were numerically higher cyclic activity rates (71.4%) in Group I, all those 'control' cows failed to become pregnant. In fact, Ovsynch protocol, as used with some modification herein, yields superior results in cycling cows [8,26], and thus the success rate may vary between the studies widely (33-77% pregnancy) [2,14-17]. Indeed, we previously observed even a considerably lower pregnancy rate of 17.5% (Ovsynch) and 21.6% pregnancy rate (Cosynch) in controls [8]. Herein, there was no pregnancy at all in controls. Apart from the predominant effect of poor management, undernutrition (poor BCS) leads to a reduced oocyte maturation and impaired embryogenesis [20,38]. This is due likely to further environmental stressors, such as unexpected/sudden changes (few times) in the content of diet given or even occasional starvations for some days. Indeed, the cows in Group IV failed to sustain their embryogenesis, presumably due further to their rather low rates of initial CL (50%).

Regarding vitamins, it is known that β-carotene is the main component of the CL synthesizing the P_4 [30]. Additionally, both vitamin D (immunostimulant) and E (antioxidant) may provide some degree of support in reproduction, but their stimulatory roles concerned are secondary to the role of vitamin A [24]. Indeed, the levels of vitamin A were markedly increased by the third samplings, but this was not the case for the levels of other vitamins given. In a previous study [8], numerically higher pregnancy rates were achieved with vitamin (β-carotene plus a-tocopherol, as precursor of vitamin E)-supplemented group, as compared to those in controls (25.8% *vs.* 17.5% for Ovsynch and 34.1% *vs.* 21.6% for Cosynch, resp.). Furthermore, vitamin A acts as immunostimulant [31], facilitating to combat the potential stress-bearing environmental factors, likely to lead uterine infections. However, as appreciated, low energy content or extended NEB pp may provoke the adverse effects of ongoing poor management conditions given. Indeed, this peculiar conditional status was visible with poorer BCS in many cows, declining from 2.56 to 2.43 units (for all) after the first AI. Undoubtedly, in a broad sense, reproduction may be blocked in underfed animals [2,20,33,37,38]. The ultimate outcome of present study was discouraging, to some extent, with low pregnancy rates obtained (8.8%, in total). Different doses/occasions of GnRH administration were used herein aiming to initiate/support the ovarian cycles further [2]. But, the overall low pregnancy results achieved may well reflect the undesirable outcome of underfeeding and/or peculiar unexpected diet changes and poor management conditions given. Indeed, sudden changes in diet composition or rapid fluctuations in feeding level and pattern of feed intake impair rumen function and metabolic homeostasis, with adverse consequences on embryonic survival [38].

Generally, it is a usual practice that, the minerals are taken daily by diet [31], since they are not expected to reside within the body. Interestingly, the levels of Zn (acting as antioxidant [38]), unlike those of others remaining virtually unchanged, increased markedly by the third samplings, regardless of treatment. However, the actual reason of this unexpected increase is unknown. In this respect, we suspect from the consumption of different source of forage (clover) possibly with higher Zn contents. Nevertheless, in a broader sense, it was observed that the minerals given (via IM route) were considerably more effective (higher CL, %) especially when they were given along with vitamins. This may indicate their synergetic effects upon the metabolism when given in combination [38-40]. Hence, diets should be supplemented with minerals and vitamins as required, especially before/during lactation.

Regarding the P_4, the results appear to be related to the initial cyclic activity rates, being higher in Group I and II, as compared to those in Group III and IV. The low levels of initial activity may also impair developmental capacities

of follicles and the CL [38]. An improved productions of oestrogen and P_4 was observed in repeat breeder cows following the Ovsynch protocol supplemented with β-carotene [26]. As expected, a higher initial cyclic activity could also sustain embryogenesis more successfully, as it was the case herein (Group II).

In the early lactation period, cows known to be in the NEB should be fed with an adequate amount of diet for productivity, health and welfare [41]. BCS lies within its immediate availability so that the management decisions could be made on the farm by a virtue of measurement of energy status. However, BCS might not always reflect the current dietary and herd statues. Hence, metabolic profiles should be determined to provide the actual herd's energy status [42,43]. In our study, BCSs have been the main criterion for the animals to be included in the study for subnormal energy balance. In addition to the BCSs, the levels of carbohydrate, lipid, protein and enzymes were also determined to reveal the metabolic profile in all animals during the study course. For this period, the levels of glucose, as well as TCOL, HDL and LDL slightly decreased in all groups, except for those in Group IV on the third samplings (for lipids). The NEB status, affecting the animals with regard to lower levels of glucose and lipids, given corresponds well with slight decreases of BCSs (0.13 unit) over the same period. The concentrations of glucose and TCOL in plasma are correlated positively with the energy balance, while the opposite was the case for creatinine, albumin and enzyme activities [42,44]. The levels of creatinine, albumin and liver enzymes remained unchanged except for a slight increase in AST level. The elevations of VLDL and TG might reflect lipolysis that involves the hydrolysis of TGs into free fatty acids during the NEB prevails. The energy statues given partially imply that the cows used herein are indeed in an underfed condition based on their metabolic profiles and BCSs. It is also noteworthy that, the effects of vitamin and mineral supplementations upon the metabolic parameters studied were negligible between the blood sampling times. Collectively, the overall trends of alterations of parameters were generally in the same manner between the experimental groups used during the study period.

Overall, the results suggest that; *i)* Ovsynch synchronisation programme supplemented with vitamins and minerals, favourably affected the plasma levels of vitamin A only, *ii)* the actual desirable effects of the supplementation were limited (apparent with vitamins) based on the luteal structure and ultimate pregnancy rates, *iii)* the levels of glucose (to some extent), protein (creatinine) and lipids (VLDL and TG) were changed by the NEB during the course of the study, and *iv)* the initial cyclic activity rates and poor BCS's (around 2.5 units) seemed to affect the overall success of synchronisation programme used in underfed dairy cows early pp.

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