



## Effect of fish meal and oil on hormone profile and reproductive variables in ewes inseminated by laparoscopy

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### ABSTRACT

The addition of n-3 polyunsaturated fatty acids (PUFA) to the diet of ruminants can benefit the reproductive process in the female. The current study was conducted to assess the effect of a short period of feeding a diet that included fish meal and oil on the progesterone ( $P_4$ ) and insulin (INS) profile, and on reproductive variables including estrous onset, pregnancy and prolificacy in virgin ewes artificially inseminated by laparoscopy (AIL). Forty-two Dorset ewes were assigned into two experimental groups: These groups were no supplementation (CON;  $n=21$ ) and a group supplemented with fish meal and oil (4 and 0.8%; FMO;  $n=21$ ). Ewes were fed the experimental diets for 15 days, beginning four days before inserting sponges for estrus synchronization and ending the day the vaginal sponges were removed. Each ewe received 0.8 kg d<sup>-1</sup> feed in individual pens. Ewes were pre-synchronized with prostaglandin  $F_{2\alpha}$  and later synchronized with chronolone sponges for 11 days. When sponges were removed, the ewes received 200 IU of eCG. The AIL began 48 h after sponge removal and estrus detection. The time of estrus onset was different among groups ( $P < 0.05$ ; CON:  $35.1 \pm 2.1$ ; FMO:  $41.0 \pm 1.8$  h). No differences were found in  $P_4$  (FMO:  $3.8 \pm 1.2$ ; CON:  $3.5 \pm 1.4$  ng mL<sup>-1</sup>) or INS concentrations in serum (FMO:  $0.12 \pm 0.02$ ; CON:  $0.13 \pm 0.03$  ng mL<sup>-1</sup>). Adding fish meal and oil to the diet did not affect pregnancy percentage (FMO: 52%; CON: 55%), but it did affect the prolificacy index (FMO: 1.63; CON: 1.25) ( $P < 0.05$ ). It was concluded that the addition of fish meal and oil to the diet of virgin ewes over a short period time delayed onset of estrus and enhanced prolificacy.

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### Contents

1. Introduction . . . . .	358
2. Materials and methods . . . . .	358
2.1. Animals and treatments . . . . .	358
2.2. Estrus synchronization . . . . .	358
2.3. Sampling and laboratory analyses . . . . .	359
2.4. Statistical analysis . . . . .	359
3. Results . . . . .	360
3.1. Estrus onset and presentation . . . . .	360
3.2. Hormone profile . . . . .	360
3.2.1. Progesterone ( $P_4$ ) . . . . .	360
3.2.2. Insulin (INS) . . . . .	360

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3.2.3. Pregnancy and prolificacy . . . . .	360
4. Discussion . . . . .	360
4.1. Estrus onset and presentation . . . . .	360
4.2. Concentrations of progesterone (P <sub>4</sub> ) and insulin (INS) in serum . . . . .	360
4.3. Pregnancy and prolificacy . . . . .	361
5. Conclusions . . . . .	361
Acknowledgements . . . . .	361
References . . . . .	361

## 1. Introduction

The positive effects of energy supplementation on reproduction are well documented in many species, but the precise mechanisms have not been clearly delineated in ewes. It is important to understand the mechanisms linking nutrition and reproduction, and to search for alternatives to improve female fertility (Hess et al., 2008).

Feeding supplemental energy during short periods can modify secretion of metabolic hormones, follicular waves and ovulatory rate on days 9–13 of the estrous cycle (Viñoles et al., 2005; Scaramuzzi et al., 2006). In particular, the addition of ingredients that contain n-3 and 6 polyunsaturated fatty acids (PUFA) to ruminant diets positively influence follicle growth, embryonic development, and cell membrane components (Heravi et al., 2007; Zachut et al., 2008).

Diets with high concentrations of n-3 PUFA are associated with low cholesterol in plasma (Robinson et al., 2002), which can have repercussions in synthesis of steroid hormones such as progesterone (P<sub>4</sub>) and estradiol (E<sub>2</sub>). Moreover, larger ovulatory follicles and corpora lutea in dairy cows that were fed these fatty acids have been reported (Petit et al., 2002; Ambrose et al., 2006; Mendoza et al., 2011). In contrast, diets high in n-6 are related to increased cholesterol, which possibly stimulates production of P<sub>4</sub> (Wathes et al., 2007).

Other studies (Thangavelu et al., 2007; Childs et al., 2008) have reported that diets supplemented with fish meal and oil as a source of n-3 PUFA (EPA, C20:5; DHA, C22:6) suppresses the synthesis of PGF<sub>2α</sub> by reducing cell membrane abundance of the fatty acid precursor arachidonic acid (ARA, C20:4, n-6), directly affecting luteum regression and favoring maternal recognition of pregnancy.

Therefore, the objective of this study was to examine the effect of targeted feeding a diet based on fish meal and oil as sources of n-3 PUFA on P<sub>4</sub> and INS profile and reproductive variables such as estrus onset, pregnancy and prolificacy in virgin ewes inseminated by laparoscopy.

## 2. Materials and methods

The study was conducted in the sheep unit of the experimental farm of the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico, complying with the guidelines for ethics and biosafety of the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1986) and the Mexican norms (NOM-062-ZOO-1999) for the use of animals in experimentation (DOF, 2001).

### 2.1. Animals and treatments

Forty-two Dorset virgin ewes of reproductive age ( $9 \pm 1.2$  months), with an average weight (AW) of  $54 \pm 4.2$  kg and a body condition (BC) of 3 on a 1 to 5 scale (Russel et al., 1969) were used.

Prior to the study, ewes were confirmed as being non-pregnant by the use of a transvaginal ecograph by ultrasound (SONOVET 600).

The ewes were randomly distributed in two experimental groups: a control group (CON;  $n=21$ ; AW =  $55 \pm 3.1$  kg; BC = 3.2) fed a diet based on corn, sorghum, soy paste and oat hay. The experimental group was supplemented with fish meal and oil (4.0 and 0.8% of total diet, respectively; Table 1; FMO;  $n=21$ ; AW =  $53 \pm 2.4$  kg; BC = 3.4). Both diets were formulated to cover the crude protein (CP: 14%) and metabolizable energy ( $10.8 \text{ MJ kg}^{-1}$ ) requirements for sheep recommended by the National Research Council (NRC, 2007).

Ewes were fed the experimental diets for 15 days (d), beginning four days before inserting sponges for estrus synchronization and ending the day the vaginal sponges were removed (Fig. 1). Ewes were housed in individual pens  $1.2 \times 2.0$  m ( $2.4 \text{ m}^2$ ) to facilitate individual feeding of  $0.8 \text{ kg d}^{-1}$  feed. After feeding they were released into shaded corrals ( $4 \text{ m}^2 \text{ ewe}^{-1}$ ) where they had free access to water.

### 2.2. Estrus synchronization

Sheep were pre-synchronized with two injections of prostaglandin F<sub>2α</sub> (65 mg chloprostenol, Celosil®, Schering-Plough) eight days apart so that all the ewes would be in the same phase of the estrous cycle. Six days after the last application, a sponge with 20 mg chronolone (Chronogest™, Intervet) was inserted into the vagina and left for 11 days. After the sponge was withdrawn, the ewes received an intramuscular injection of 200 IU of equine chorionic gonadotropin (eCG, Folligon™, Intervet).

Estrus was detected 24 h after sponge removal by rams fixed with an apron. After ram introduction, estrus behavior was

**Table 1**

Nutrient content in the experimental diets supplemented with fish meal and oil (FMO) and control group (CON).

Ingredients (% DM <sup>a</sup> )	Experimental diet	
	CON	FMO
Corn	25.09	33.64
Sorghum	10.15	10.13
Soy paste	13.75	7.43
Fish meal	-	4.01
Fish oil	-	0.84
Oat hay	44.46	37.40
Molasses	4.76	4.75
Mineral mix <sup>b</sup>	1.79	1.80
<b>Determined analysis (%)</b>		
Crude protein	14.45	14.20
Total lipid	4.32	5.86
Metabolizable energy (MJ kg <sup>-1</sup> )	10.93	11.05
Calcium	0.42	0.45
Phosphorus	0.31	0.36

<sup>a</sup> DM: Dry matter. <sup>b</sup> Mineral mix: phosphorus 10%, calcium 12%, iron 0.5%, magnesium 0.1%, copper 0.15%, zinc 0.12%, manganese 0.055%, cobalt 0.05%, iodine 0.02%, selenium 200 ppb, Vitamin A 50000 UI.

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