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# Effect of Feeding High Urea Diets on Metabolites, Hormones and Ionic Composition of Follicular Fluid in Camels

M.A. Alfattah, A.I. Al-Mubarak, T.A. Althnaian, I.F. Albokhadaim,
A.K. Al-Haider and A.M. Homeida
Camel Research Centre, King Faisal University, P.O. Box 1757, 31982 Al-Ahsa, Saudi Arabia

**Abstract:** It is now established practice to upgrade the protein content of low quality roughage by urea treatment. Administration of urea may result in some adverse effects. This study was carried out to investigate the effects of feeding urea-treated wheat straw diet on the hormonal and metabolic environment of ovarian follicles. Feeding of urea-treated wheat straw to camels resulted in significantly increased concentration of serum and follicular fluid urea compared to their counterparts that fed straw only. The increased urea in follicular fluid was accompanied by decreased concentration of estradiol 17 β and progesterone and increased activity of lactic dehydrogenase in the fluid. This suggested that feeding of high urea diets may affect follicular fluid composition and development of oocyte.

**Key words:** Urea diets, metabolites, hormones, ionic composition, follicular fluid, camels

## INTRODUCTION

Wheat straw is the most abundant agricultural animal in Saudi Arabia. It is widely used as cheap source of bulk feed to ruminants. The feed value of wheat straw is low in protein, minerals and vitamins (Saadullah *et al*., 1981). It is now established practice in some parts of the world to upgrade the protein content of low quality roughage by urea treatment (Al-Shami and Al-Sultan, 2006). Urea treated straw was reported to be superior to untreated straw in terms of digestability, intake of nutrients (Pathak *et al*., 2005) and milk production (Majumdar *et al*., 2003). However, adverse effect may occur due to consumption of large amount of urea.

A negative relationship between high milk urea level and fertility performance in dairy cattle is reported widely (Carroll *et al*., 1994; Butler, 1998).

The follicular fluid forms the biochemical environment of the oocyte before ovulation (Jozwik *et al*., 2001). Follicular fluid is in part an exudate of serum and in addition is partially composed of locally produced substances which are related to the metabolic activity of follicular cells (Armstrong *et al*., 2001).

This metabolic activity, together with the barrier properties of the follicular wall is changing significantly during the growth phase of the follicle (Wise, 1987; Gosden *et al*., 1988). This study was designed to investigate ovarian follicular metabolic environment following feeding of urea-treated straw to camels.

## MATERIALS AND METHODS

**Urea treatment of wheat straw:** About 1 ton of wheat straw (tibin) was treated with 4% w/w urea (Saudi Basic Industries Corportion) solution (12.5%) and subjected to urealysis by anaerobic storage for 8 weeks. This treatments of urea was expected to increase the crude protein content of straw from 3% in straw to 8% in urea treated straw (Al-Shami and Al-Sultan, 2006).

**Experimental animals and treatments:** Twelve mature (2-4 years old) and weigh 300-400 kg body weight female camels were used in the study. They were divided equally into 4 groups.

**Group 1:** Animals were given wheat straw as a sole diet for 2 months. Water and mineral salt licks were provided *ad libitum*.

**Group 2:** Animals were treated similar to group 1 but in addition were given each 1 kg urea-treated straw and 2 kg of concentrates.

**Corresponding Author:** M.A. Alfattah, Camel Research Centre, King Faisal University, P.O. Box 1757, 31982 Al-Ahsa, Saudi Arabia

**Group 3:** Animals were treated similar to group 1 but in addition were given each 2 kg urea-treated straw and 2 kg of concentrates.

**Group 4:** Animals were treated similar to group 1 but in addition were given 4 kg of urea treated straw and 2 kg of concentrate.

**Ovary collection and sample preparation:** On the last day of feeding period ovaries were collected immediately after slaughter and a blood sample was taken during exsanguinations.

Both ovaries and the blood sample were identified by using eartag number of the camel. Blood is allowed to coagulate for 20 min at 15°C and then cooled at 4°C, after which the ovaries and blood samples were transported on ice (4°C) to the laboratory. Serum was separated and stored at 20°C until analysis.

Ovaries were washed twice in cooled 0.9% NaCl (4°C) and blooted dry. Two different follicle classes, based on follicle diameter were considered for puncture; small follicles (<4 mm) and large follicle (>10 mm). Follicular fluid was collected by aspiration with a 26G needle and 1 mL syringe and pooled per follicle class within camel.

**Biochemical analysis:** In each sample, the concentrations of sodium, potassium, glucose, lactic dehydrogenase, urea and total protein were measured. The determination of metabolite levels in follicular fluid and blood serum is done using wet chemistry technique on clinical chemistry autoanalyser (Hitachi 911, Japan) using commercial kits. Progestrone and esrtadiol 17 β were estimated by radioimmunoassay methods (Homeida *et al*., 1988).

## RESULTS AND DISCUSSION

Feeding of urea treated straw resulted in significantly ($p<0.05$) increased concentration of plasma urea in group 2-4 animals compared to group 1. Other researchers also reported that dietary urea supplementation increases the urea concentration in blood of camels (Homeida and Al-Shami, 2009) and lactating cows (Broderick *et al*.,1993).

Feeding increasing amount of urea has also resulted in an increased concentration ($p<0.001$) of urea in follicular fluid (Table 1).

The follicle basement membrane is very permeable to low and high molecular substances that can diffuse into follicular fluid in a matter of minutes (Payer, 1975; Kamarianos *et al*., 2003). The effect of increasing concentration of follicular urea was accompanied by nonsignificant differences in groups 2-4 for protein, glucose, Na and K ions (Table 1).

However, estradiol and progesterone concentration was significantly decreased. Exposure of bovine granulosa cells invitro to organic compounds at high concentration resulted in significant decrease in estradiol and progesterone secretion (Faundez *et al*., 1996; Kamarianos *et al*., 2003).

In most mammalian species intrafollicular levels of steroids reflect the physiological status of follicles (Gerard and Monget, 1998). The activity of Lactic Dehydrogenase (LDH) was increased in follicular fluid of group 4 animals (Table 1). Increased LDH activity has been observed in a variety of conditions as a result of tissue necrosis.

Increased activity of LDH in follicular fluid may indicate early follicular degeneration (Wise, 1987) as in the case of follicular atresia (McNatty, 1981).

Table 1: Mean±SD concentrations of ions steroid hormones and metabolites in follicular fluid of camels given various urea diets

| | Group 1 | | | Group 2 | | | Group 3 | | | Group 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | Serum | Small follicle | Large follicle | Serum | Small follicle | Large follicle | Serum | Small follicle | Large follicle | Serum | Small follicle | Large follicle |
| Estradiol 17 β (ng $mL^{-1}$) | 0.02±0.003 | 17.2±2.8 | 67.3±4.3 | 0.03±0.002 | 16.2±2.6 | 62±3.9 | 0.02±0.003 | 15.3±7.5 | 66±4.2 | 0.02±0.003 | 10.2±1.6* | 42 ± 2.6* |
| Progesterone (ng $mL^{-1}$) | 0.12±0.003 | 15.1±2.2 | 31.2±2.6 | 0.10±0.002 | 14.2±2.3 | 30.1±2.5 | 0.11±0.003 | 13.2±2.1 | 30.4±2.6 | 0.13±0.002 | 9.1±1.9 * | 22±1.5* |
| Glucose (µm) | 4.61±0.12 | 1.93±0.11 | 3.6±0.17 | 4.51±0.21 | 1.87±0.12 | 3.4±0.15 | 4.42±0.31 | 1.85±0.2 | 3.2±0.16 | 4.60±0.22 | 1.94±0.12 | 3.2±0.25 |
| Urea (µm) | 4.1±0.21 | 4.71±0.25 | 4.2±0.22 | 6.4±0.24 | 7.3±0.26 | 7.7±0.25 | 11.2±1.2* | 12.2±1.5* | 11.3±1.4* | 20.2±2.1* | 22.1±2.2 | 23.2±2.4* |
| Total protein (g $dL^{-1}$) | 6.7±0.12 | 6.2±0.11 | 6.3±0.17 | 6.8±0.13 | 6.3±0.14 | 6.5±0.15 | 6.6±0.14 | 6.4±0.15 | 6.6±0.13 | 6.8±0.15 | 6.6±0.14 | 6.8±0.15* |
| Lactic dehydrogenase (u $mL^{-1}$) | 96±6.1 | 3.1±0.2 | 2.33±0.21 | 91±6.2 | 3.4±0.3 | 3.45±0.21 | 90±6.6 | 5.2±0.22 | 8.3±0.62 | 94±5.9* | 16.6±0.51* | 22.3±2.1* |
| Na (µm) | 141±3.4 | 137.2±2.6 | 136.5±3.1 | 138±4.1 | 127±4.2 | 129±5.2 | 132±5.1 | 128±4.2 | 125±3.7 | 134±3.6 | 122±4.5 | 124±5.5 |
| K (µm) | 5.2±0.1 | 10.2±0.25 | 6.1±0.21 | 5.4±0.2 | 11.2±0.32 | 6.7±0.24 | 5.5±0.2 | 11.1±0.33 | 6.6±0.23 | 5.3±0.3 | 10.9±0.3 | 6.3±0.22 |

*$p<0.05$ significantly different from their respective values of group 1

## CONCLUSION

Since, the ovarian follicular fluid constitutes the micro-environment in which the oocyte develops and finally matures and the fluid is partly an exudate of serum that surrounding a permeable cell layers, it may be of particular importance to safe gurd against the use of substances with potential reproductive toxicity in the follicular fluid because the oocyte completes maturation before ovulation within this milieu.

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