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EFFECT OF DIETARY ENERGY ON RESPONSE OF BUFFALOES WITH SMOOTH INACTIVE OVARIES TO GONADOTROPHIN RELEASING HORMONE INJECTION

(With 3 Tables)

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تأثير مستوى الطاقة في العليقة على استجابة الجاموس الذى يعانى من خمول المبايض لحقن الهرمون المحرر للحاثة المنسلية (الجي إن أر أتش)

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لدراسة تاثير مستوى الطاقة في العليقه على استجابة الجاموس الذي يعاني من خمول في نشاط المبايض لحقن هرمون الجي إن أر أتش. تم إجراء هذه الدراسة على عدد ٢٤ جاموسه تعانى من خمول في نشاط المبايض بعد ٧-٨ شهور من الولادة. كانت هذه الحيوانات تغذى على عليقه تفي باحتياجاتهم من الطاقة وتزيد في البروتين والأملاح والألياف قبل بدء التجربة. تم تقسيم هذه الحيوانات الى مجموعتين رئيسيتين بكل منها عدد ١٢ جاموسة إحداهما ضابطة (تلك التي استمرت على العليقه المقدمة قبل التجربة محتوى مجموع المواد الغذائية المهضومة بها ٥٧,٧%) والثانية غذيت على عليقه تم رفع فيها مجموعة المواد الغذائية المهضومة إلى ٧١,٣%. بعد شهر من بدء التجربة تم تقسيم كل مجموعة رئيسية الى مجموعتين (٦ جاموسات لكل مجموعة) إحداهما ضابطة والثانية تم حقنها بهرمون الحى إن أر أتش (٥ مل ريسبتال). لمتابعة استرجاع المبايض لنشاطها تم فحص المبايض عن طريق المستقيم وكذلك مراقبة وقوف الحيوانات لوثب الطلوقة عليها. تم جمع عينات دم أسبوعيا وذلك لقياس تركيز كل من هرمون الانسولين والجلوكوز واليوريا في البلازما. وجد ان رفع مستوى الطاقة في العليقه أدى إلى زيادة استجابة الجاموس (استعادة نشاط المبايض) لحقن هرمون الجى إن أر أتيش من ٣٣.٣٣% إلى ١٠٠%. وقد لوحظ ان زيادة مستوى الطاقة في العليقه أدى إلى زيادة معنوية في تركيز الجلوكوز والانسولين في البلازما. وقد كانت هناك زيادة معنوية في اليوريا في مصل الجاموس المغذى على العليقه الضابطة في مستوى الطاقة. ومن هذه الدراسة يمكن استخلاص انه يجب رفع مستوى الطاقة في عليقه الجاموس الذي يعانى من خمول نشاط المبايض قبل علاجها بحقن هرمون الجى إن أرأتش.

SUMMARY

Effects of energy status of buffaloes with ovarian inactivity on their response to gonadotrophin releasing hormone (GnRH) injection were studied using 24 anoestrus buffaloes suffering from ovarian inactivity. The animals were allotted into two main groups (12 each) standard or high energy diet group. For GnRH injection, each main group was allotted into two subgroups (6 each) non injected and injected subgroup. Resumption of ovarian activity is detected weekly by rectal examination of ovaries as well as by bulls. Blood samples were collected weekly for determination of blood glucose, insulin and urea nitrogen. Increasing the energy density increased the response to GnRH injection from 33.33% to 100%. Also increasing the energy density increased ($P<0.05$) plasma glucose and insulin concentrations and decreased ($P<0.05$) plasma urea nitrogen. It could be concluded that high energy intake improves the response of buffaloes with inactive ovaries to GnRH injection.

Key words: *Dietary Energy, Inactive Ovaries*

INTRODUCTION

Ovarian inactivity is the most important cause of infertility in Egyptian buffaloes. Hormonal treatment of ovarian inactivity in buffaloes suffering nutritional deficiencies and/or imbalance especially those kept by farmers who are not aware by the principles of sound nutrition is less effective. The efficacy of gonadotrophin releasing hormone (GnRH) in initiating cyclicity in buffaloes appears to be dependent on the follicular status at the time of injection (Palta and Madan, 1995). The development of follicles to the pre-antral stage is not gonadotrophin dependent solely (Hafez, 1987) and the emergence of dominant follicle with greater steroidgenic output and ovulation rate (Stephen and Butler, 1997) is related to energy status of the animal (Murphy et al., 1991; Bergfeld et al., 1994 and Khireddine et al., 1998). With energy restriction, follicular development is impeded; the quality of the produced ovum is reduced (Lucy et al., 1991) and even the reproductive function ceases (Randel, 1990).

The energy status of the animal affects its reproduction through two folds, the first is being on the level of the ovary and follicular development and this effect is modulated through the glucose homeoresis which guarantees a normal insulin level in blood (Studer, 1993). Insulin has been found to be effector of granulosa cells (Stephen and Butler, 1997). Khireddine et al., 1998 found a positive correlation between plasma insulin and the size of the largest follicle. It stimulates the proliferation of the granulosa cells which synthesize growth factors (Maruto et al., 1993) that stimulate the dominance of ovarian follicles (Murphy et al., 1991). Afterwards, it also increases the sensitivity of the granulosa cells to FSH stimulation (Bergfeld et al., 1994), expresses LH receptors and stimulates steroidogensis (Grimard et al., 1996). On the level of the hypothalmus-pituitary axis, it has been reported that energy restriction depressed GnRH release in the hypothalmus (Butler and Smith, 1989 and Lucy et al., 1991) which in turn affects the LH release from the pituitary gland (Randel, 1990; Lucy et al., 1991, and Rhodes et al., 1995).

This study was designated to investigate the effects of dietary energy density on the response of buffaloes suffering ovarian inactivity to GnRH injection.

MATERIAL and METHODS

1. Buffaloes and diets:

Twenty-four anestrus Egyptian buffaloes, suffering ovarian inactivity, 7-8 months postpartum, aged 6-8 years, and had good general healthy condition were selected (from a buffalo herd in a private farm at El-Gharbia province) upon two successive rectal examinations of ovaries at 10 days interval to carry out this study. Prior to the start "7-weeks experimental period" (from March to the end of April) buffaloes were individually fed 13 kg dry matter (DM) daily of berseem, wheat straw, and trace-mineralized salt diet (Table 1). The total digestible nutrients (TDN) content of this diet was 57.7%, (standard energy level), whereas protein, fiber and calcium levels were above the requirements recommended by Ranjhan and Pathak, 1982 for buffaloes weighed 550 kg and produced 6 kg milk of 7.3% fat daily. Buffaloes were allotted by body condition score and pre-experimental milk yield into two main groups (12 each), standard energy level (continually fed the pre-experimental diet, 57.7% TDN) and high energy level diet group (71.3% TDN). Elevated TDN content of high energy diet was obtained by addition of concentrate mixture (prepared from corn grain, wheat bran, dried beet plup, SBM, whole cottonseed, dried fat) to the standard diet in replacement of berseem and wheat straw at a rate of 65.86% of DM (Table 1). Diet of high energy group was formulated to be isonitrogenous with the standard energy one (Table 1). Dietary ingredients were supplemented with mineral supplements to be similar (Table 1). Dry matter intake for standard or high energy diet was maintained at 13 kg daily throughout the experiment. Buffaloes on standard diet offered wheat straw at 6 h a.m. and 5 h p.m. whereas berseem offered at 7 h a.m. and 6 h p.m. While those on high energy diet fed the concentrate mixture with wheat straw at 6 h a.m. and 5 h p.m. whereas berseem offered separately at 7 h a.m. and 6 h. p.m.

2. GnRH injection and follow up:

The buffaloes were examined rectally once weekly and observed twice daily for oestrus behaviour as well as standing for bull to be mounted. After 3 weeks of the previously described feeding regime, 6 buffaloes from each main group were injected by GnRH. Analogue Busereline [Receptal®]. Each buffalo was injected intramuscular by 5 ml Receptal® containing 0.02 mg Busereline. The remaining 12 buffaloes 6 from each main group were left untreated (control). The resumption of ovarian activity is indicated by presence of either Graffiaan follicle followed by corpus luteum or and/or corpus luteum. Expression of bellowing, decreased milk yield and hyperemia of both vulva and vagina are suggestive signs of oestrus which are assured by standing for bull to be mounted.

3. Sampling and laboratory procedures:

Two samples of berseem were composited weekly for DM determination in a forced-air oven at 60°C (48 h) to facilitate weekly adjustment of berseem content of as-fed. Dietary samples were composited and ground after oven dried. Subsamples of berseem, concentrates, and wheat straw were analyzed for crude protein (CP), ether extract (EE) according to A.O.A.C. (1980) procedures. Neutral Detergent fiber (NDF) and Acid Detergent Fiber (ADF) were determined by the procedure of Goering and Van Soest (1970). Calcium (Ca) and phosphorus (P), were determined colorimetrically according to Elveback, 1970 for Ca, and Goldenbrig, 1966 for P. Total digestible nutrients (TDN), nonstructural carbohydrates (NSC), ruminal degradable protein (RDP) and ruminal undegradable protein (RUP) were calculated using NRC (1989) values.

Blood samples were collected weekly via acute jugular puncture into evacuated heparinized tubes 2 h after morning feeding. Plasma was separated immediately by centrifugation then stored at -20°C until assayed by enzymatic colorimetric methods for determination of glucose (Waner *et al.*, 1970) and urea nitrogen (Coulombe and Favreau, 1963). Plasma insulin was quantified by radioimmunoassay as described by McGuire et al., 1995 for bovine plasma.

4. Statistical analyses:

Data collected during the test period were subjected to ANOVA using the General Linear Models Procedure (GLM) SAS (1985). Duncan's multiple range test was applied to test the significance between the two treatments (SAS).

RESULTS

1. Diet composition:

Nutrients composition of the experimental diets are shown in Table 1: The diets were isonitrogenous (14.6% CP). The degradability of protein differed (76.2% for standard versus 66.4% for high energy diet) this may be due to high degradability of protein in berseem (NRC 1989) which is the main component of the standard diet. Calculated TDN of the standard diet was 57.7% which is adequate and met energy requirements of a buffaloes weighed 550 kg and produce 6 kg milk of 7.3% fat daily (Ranjhan and Pathak, 1982). Addition of concentrate at a rate of 65.86% of DM raised TDN content to 71.3%, fat to 4.3%, nonstructural carbohydrate (NSC) to 33.3%, RUP to 33.6% and decreased NDF to 36.9% and ADF to 21.9%. Calcium and phosphorus contents were similar in both diets.

2. Resumption of cyclicity and response rate:

Investigation of Table 2, showed that increasing the energy density increased the resumption of ovarian activity from zero to 66.7% and improved the response of buffaloes to GnRH injection from 33.33% to 100%.

3. Blood metabolites and insulin concentrations:

Concentrations of urea nitrogen, glucose and insulin in plasma of buffaloes fed the standard and high energy diets (GnRH injected or non-responded or not) are summarized in Table 3. The concentration of plasma urea nitrogen was increased ($P < 0.05$) in buffaloes consumed the standard energy diet than those on high energy diet (59.14 versus 44.12 mg/dl), whereas the concentrations of glucose (56.91 vs 54.66 mg/dl) and insulin (10.35 vs 2.00 μ IU/ml) were significantly ($P < 0.05$) higher in plasma of buffaloes fed high energy diet.

DISCUSSION

The resumption of ovarian activity in buffaloes fed high energy diet had the highest response to GnRH injection. Similar results with beef cattle were reported by Leers-Sucheta et al., 1994. This may be attributed to the fact that the high energy diet contains synchronized concentrations of nonstructural carbohydrates (33.3%) and ruminal degradable protein (66.4%), which is similar to NRC (1989) recommendation for lactating cows, to obtain optimal ruminal fermentation. Sufficient nonstructural carbohydrates (sugars and starches provided by grains) provide an adequate energy in the form of propionate (Elliot et al., 1995; Gummer, 1995). In the liver, propionate is converted into glucose (Annison and Armstrong 1970). Elevated blood glucose increases insulin release (Studer et al., 1993; Martin and Elliot, 1997). High levels of blood glucose and insulin affects directly on the ovary, stimulating the emergence of dominant follicles (Murphy et al., 1991). The greatest and largest follicle which potentially ovulate is found in the cows in highest energy balance (Lucy et al., 1991; Stephen and Butler 1997 and Khireddine et al., 1998). On this line, Palta and Madan, 1995 found that the initiation of oestrus cyclicity in buffaloes appears to be dependent on the follicular status at the time of GnRH treatment. In support, Cox et al., 1994 reported that the stoppage of insulin treatment of gilts suffering diabetes during the period of preovulatory follicular growth resulted in reduced follicular growth, lowering in both peripheral and intra-ovarian IGF_1, abnormalities in follicular steroidgensis and increased level of macroscopic atresia, they also added that these changes are independent of circulatory gonadotrophins. At the same time these findings explained lower response of buffaloes fed the standard energy diet to GnRH injection in this experiment which may be attributed to lower blood glucose and insulin levels (Table 3). Besides higher blood glucose and insulin concentrations increased the capability of pituitary to respond to GnRH injection in terms of LH and FSH release (Randel, 1990; Lucy et al., 1991 and Rhodes et al., 1995). Insulin will augment the effect of FSH on the granulosa cell (Amsterdam et al., 1988; Bergfeld et al., 1994) and consequently increasing the number and affinity of LH receptors at follicles (Amsterdam et al., 1988; Grimard et al.. 1996). On the other hand, low response of buffaloes fed the standard energy diet to GnRH injection may be attributed to high RDP: Low RUP ratios is that such diet contains insufficient NSC (Table 1) for rumen microbes to fully utilize the free ammonia nitrogen produced from RDP (NRC, 1989) which drives up the level (Table 3) of plasma urea nitrogen

(Folman et al., 1981; Canfield et al., 1990 and Kaim et al., 1993). Elevated blood urea nitrogen having an adverse effect on hypophyseal pituitary ovarian axis (Jordan and Swanson, 1979; Visek, 1984; Blauweikel et al., 1986; Ferguson et al., 1986 and Ferguson and Chalupa, 1989).

In conclusion, this study revealed that, high energy intake increased blood glucose and insulin and decreased urea nitrogen concentrations; a condition which favours high response of buffaloes with smooth inactive ovaries to GnRH injection.

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Table 1: Ingredient and nutrient composition of standard and high energy diet.

Composition	Diet	
	Standard energy	**High energy**
Ingredients	% of DM	
Berseem	79.20	29.00
Wheat straw	19.20	1.53
Concentrate Mix[1]	-	65.86
Limestone	-	3.00
Sodium monobasic phosphate	1.14	-
Common salt	0.23	0.38
Micro-minerals mixture[2]	0.23	0.23
Nutrient		
Analyzed composition		
DM %	32.34	69.19
	% of DM	
CP	14.60	14.60
EE	1.40	4.30
NDF	56.70	36.90
ADF	42.10	21.90
Ash	12.50	10.90
Ca	2.00	2.00
P	0.46	0.45
Calculated composition		
RDP (% of CP)[3]	76.20	66.40
RUP (% of CP)[3]	23.80	33.60
NSC[4]	14.80	33.30
TDN[5]	57.70	71.30

[1] Concentrate mix: Prepared from 35.04% ground yellow corn; 26.42% wheat bran; 21.03% dried beet plup; 11.68% soybean meal; 3.49% whole cottonseed; 1.17% protected fat; 1.17% sodium bicarbonate (on DM basis).

[2] Each 230 g of micro-mineral mixture contains: 177.28 g calcium carbonate; 18.18 g zinc sulfate hepatahydrate; 15.625 g ferrous sulfate monohydrate; 14.81 g manganese sulfate; 3.92 g copper sulfate pentahydrate; 0.085 g potassium iodide; 0.05 g cobalt sulfate pentahydrate; 0.05 g sodium selenite. The micro-mineral mixture was formulated to provide the following per kilogram diet 40 mg Zn; 50 mg Fe; 40 mg Mn; 10 mg Cu; 0.6 mg I; 0.1 mg Co and 0.3 mg Se. The prepared micro-mineral mixture was thoroughly mixed with concentrate portion of the diet for high energy and with wheat straw for standard energy group.

[3] Estimated value from NRC (1989)

[4] Nonstructural carbohydrate = 100-[CP% + EE% + Ash % + NDF%]

[5] Estimated value from NRC (1989).

Table 2: Effect of dietary energy on response of buffaloes to GnRH injection.

Energy level / GnRH Injection	Standard			High		
	No. of animals	Responded		No. of Animals	Responded	
		No.	%		No.	%
Non injected	6	Zero	Zero	6	4	66.7
Injected	6	2	33.33	6	6	100

Table 3: Concentrations of urea nitrogen, glucose, and insulin in plasma of buffaloes fed standard and high energy diets.

Item	Diet	
	Standard energy	High energy
Urea nitrogen (mg/dl)[1]	59.14 ± 0.43 *	44.12 ± 0.37
Glucose (mg/dl)[2]	54.66 ± 0.49	56.91 ± 0.37*
Insulin (μ IU/ml)[1]	2.00 ± 0.01	10.35 ± 0.07*

[1]n = 72
[2]n = 71
Means ± Standard error are significantly differed at $P < 0.05$.