Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 1172-1176

Received: 03-01-2018
Accepted: 04-02-2018

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Effect of variable dietary energy levels on reproductive hormonal profile, body condition score, body weight and blood metabolites concentration of early lactating cows

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Abstract
The aim of the study was to determine the effect of different dietary energy levels on dry matter intake, reproductive hormonal profile body weight, condition score and blood metabolites of early lactating Friesian cows. Nine early lactating cows of nearly the same body weight (410±10) and score (2.70±0.10) were selected from the university dairy farm and randomly divided into three groups with three animals in each group under completely randomized design. Ration were formulated being iso-nitrogenous (CP 16%) and different in dietary Metabolizable energy (ME) levels, viz; (i) NRC100% (2.75Mcal/kg ME),(ii) NRC(-12)% low ME (2.57Mcal/kg) and (iii) National Research Council (NRC +12%) high (2.93Mcal/kg). Significantly higher dry matter intake (P<0.05) was recorded for group +12% ME (13.78±0.17kg/day), followed by NRC 100% (13.14±0.12 kg/day) and NRC -12 was (12.68±0.11kg/day) recorded. Loss in body weight was decreased (p<0.05) with increasing dietary ME level in the diet from 2.57 to 2.93Mcal/kg ME and overall gain in body weight and condition score was improved in group NRC +12% than other groups. Results of blood metabolites show that blood glucose level was significantly increased with increased in energy level whereas blood urea nitrogen levels showed (p<0.05) decrease with increase in energy level of ration. It was concluded that diets containing high dietary energy (12%) above than the NRC recommended level for early lactating cows conferred in an increase in intake, improved the body score, body weight gain and managed the optimum level of blood metabolites which may result good health, fertility and productivity parameter of dairy cattle.

Keywords: dry matter intake, metabolizable energy, body score, blood metabolites

1. Introduction
Livestock farming is essential for the supply of meat, milk and also a source of additional income for small farmers and livestock owners in Pakistan. Livestock contribution to the national GDP is almost 11.9% and 55.9% to the agriculture share [1]. During early lactation, the dietary energy requirements of high yielding dairy cows for maintenance and milk production exceed the amount of energy obtained from dietary sources. Thus, the high energy requisite at the onset of lactation results in a negative energy balance (NEB) that begins a few days before calving and usually reaches its maximum to two weeks postpartum, which may negatively affect health, production and fertility after parturition [2, 3]. The productivity of cows is below their genetic potential, which is due to various factors like poor breeding policies, traditional system of raising animals either by grazing or crop residues, providing green fodder with or without any supplementation, no group feeding system, inadequate and imbalance feeding based on farmer myth, poor management and prevalence of diseases. Beside these, scarcity and fluctuation of the quality and quantity of animal feed supply throughout the year is another factor that causes low productivity of dairy cows in the country [4]. In Pakistan, dairy cows are mostly fed on low quality roughages and crop residues, which are poor in energy, protein, minerals and vitamin contents [5]. These feeds are not efficient in nutrient like energy and protein to full fill the nutrient requirement of animals for production and reproductive activity.
Within nutritional requirement of dairy cows, energy is one of the most critical nutrients which may affect production performance in early lactation. Earlier studies [6, 7] reported that sufficient dietary energy is an important factor in lactating animals which may prevent

negative energy balance and other metabolic disorders. To reduce the gap of energy requirement and intake dairy cows rely on body reserves that were stored in the last stage of lactation. Gained in body tissue fats in large quantities and overfeeding of dairy cows during the gestation period along with the need for high milk to mobilize larger quantities of body reserves in lactation lower dry matter intake [8]. Feeding Balanced and according to requirement of the cows in late gestation increases feed intake postpartum and decreases mobilization of body reserves and loss in body weight [9]. Body Lipid mobilization and reserves makes a considerable contribution to the energy requirements for milk production in early lactation and undergoes metabolic changes, due to which animal loses weight and body condition score [4].

Reduction in milk production, increase in occurrence and severity of metabolic disorder, like fatty liver, ruminal acidosis and ketosis and a decrease in reproductive performance closely associated with Negative energy balance in postpartum cows [10]. The physiological changes which are associated with NEB are: reduced circulating concentrations of insulin and IGF-1; delayed resumption of ovarian activity [11], more days till first postpartum estrus observed, more days till first ovulation, decreases LH pulse frequency, reduce growth rate and diameter of the dominant follicle, decrease weight of corpus lutium, reduce peri-estrus hormone like estradiol (E2), Progesterone, lower conception and pregnancy rate, increase more days open and ultimately increase calving intervals and possible negative effect of the metabolites NEFA and BHB (beta hydroxyl butyrate), together with low level of circulating glucose concentrations on oocyte development [12, 13].

Several nutritional management strategies have been proposed to increase energy intake during early lactation. Feeding high quality forages, an increase in dietary energy concentration of ration, increasing the concentrate: forage ratio, or adding supplemental fat to diets are some of the most common ways to improve energy intake in cows and prevent weight loss and body condition. Therefore the current study was designed to study the effect of three different dietary energy treatment of early lactating dairy cows for body condition score, change in body weight, reproductive hormones and blood metabolites of early lactating cows.

2. Materials and Methods

2.1 Animal selection and diet formulation: Nine early lactating multiparous Holstein Friesian cows (BW 400±10kg), Body condition score BCS (2.70±0.10) were selected for the experiment from the University of Agriculture Peshawar Dairy Farm Malakandaire, Khyber Pakhtunkhwa Peshawar. These animals were randomly divided into three groups consists of three cows/group NRC 100, NRC +12 and NRC -12 under completely randomized design. The animals were assigned to three dietary treatments one was NRC recommended level (I) (2.75 Mcal/kg ME) and (II)12% low ME (2.57 Mcal/kg) and (III)NRC(12%) high ME (2.93 Mcal/kg) diet then NRC (2001) recommended level for lactating cows. The total mix ration (TMR) diets were formulated as Iso-nitrogenous (CP 16%) and diets were fed twice daily (morning and evening) to individual animals and the feed refused were measured the next morning to determine daily feed intake expressed in (DMI), the experiment lasted for seventy-four days. Weight of all animals were recorded by digital weight balance for changes in live body weight and body condition score were measured fortnightly by the method of [11]. Concentrates and berseem samples were collected for proximate analysis and were performed in the Laboratory of Animal Nutrition according to Association of Official Analytical Chemists [14]. Ingredients and percentage of each ingredient is total mix ration is given in table 1.

2.2 Blood collection and laboratory analysis: Blood samples were taken from all groups of animals on each fifteen day after start of experiments just after feeding in a sterile syringe from jugular vein throughout the experiment. After collection blood samples were kept at room temperature to clot. Serum was collected through centrifugation of samples at 1500 rpm for 20 minutes and stored at -20°C in refrigerator until analysis [15]. Serum glucose and serum urea nitrogen were determined by spectrophotometer in laboratory of Histopathology in Department of Animal Health. Commercial ELISA kit (BioCheck USA, INC 323 Vintage Park Drive Foster City, CA 94404) was used for determination of serum LH. For measurement of serum progesterone concentration BioCheck ELIZA kit with the (USA, INC 323 Vintage Park Drive Foster City, CA 94404) was used.

2.3 Statistical analysis: Research Data was collected and maintained in Microsoft office. Data were analyzed by using the GLM procedure (SAS, Version 8.02) as described by [16]. Effects of factors were declared significant at $P<0.05$.

3. Results

3.1 Feed intake and body condition score: The effect of different dietary energy levels on dry matter intake, body condition score, change in body weight and blood metabolites like blood glucose and serum urea nitrogen of early lactating Holstein Friesian cows are given in Table 2. Mean dry matter intake of the dairy cows was increased significantly ($p<0.05$) with increasing energy concentration in the diet. High DMI (13.78 kg/day) was observed for NRC +12 which was high energy (2.93Mcal/kg) followed by treatment NRC 100 (13.14) and NRC -12% were (12.68 kg/day).Loss in body weight was significantly decreased ($p<0.05$) with increasing dietary energy level in diet from 2.57 Mcal/kg to 2.93Mcal/kg ME. The overall high loss in body weight was recorded at group B was (21.00±0.57kg/cow) where as high energy diet improved body weight gain. The diet significantly increased the body condition score throughout the experimental period of 75 days and highest BCS (2.75±0.81) was recorded for NRC +12 followed by NRC 100 and NRC -12% having BCS (2.64±0.00) and (2.59±0.00) respectively given in table 1. Changes in body weight depend on DMI and energy content of diet because after parturition feed intake of cows decrease due to which animal loss body weight and condition very quickly whereas high weight gain for high energy diet.

3.2 Blood glucose, Urea nitrogen and reproductive hormones: Higher blood glucose level (53.00mg/dl) was recorded in the group NRC +12 followed by group NRC 100 and NRC -12% were 49.47and 47.33mg/dl respectively. High BUN (16.6±0.22) was recorded at group NRC -12% while low BUN (13.1±0.05 mg/dl) was recorded in diet NRC +12 groups given in table 1. Progesterone is an important hormone in reproductive process including puberty, resumption of estrous cycles, establishment and maintenance of pregnancy. Progesterone concentration was significantly increased with high energy diet from 0.16 to 0.73 ng/ml. Progesterone level was lower in early lactating dairy cows because P4 level decrease very quickly soon after parturition and remains at a

low level and undetectable until initiation of estrous cycle or formation of CL after first ovulation. Increasing dietary energy in ration significantly increased serum luteinizing hormone. The overall mean LH profile was significantly higher (0.75ng/ml) in group C compared to group A and B 0.17 and 0.16 ng/ml respectively.

Table 1: Ingredients and nutrient composition of experimental ration

Ingredients	A (100%)	B (88%)	C (112%)
Cotton Seed Cake	06	12	04
Maize oil cake	04	04	11.2
Wheat Bran	18	18	7.2
MaizeGlutton (20%)	04	3.2	08
Maize grain	04	00	06
Mustard seed cake	3.2	02	2.8
DCP	0.8	0.8	0.8
Berseem	40	40	40
Wheat Straw	20	20	20
Total	100	100	100
CP%	16	16	16
ME Mcal/kg	2.75	2.57	2.93

CP: crude protein, DCP: di calcium phosphate,

Table 2: Effect of different dietary energy levels on dry matter intake body condition score change in body weight, reproductive hormones and blood metabolites of early lactating Holstein Friesian cows.

Ingredients	NRC (100%)	NRC (88%)	NRC (112%)
DM intake (kg/day)	13.14±0.12 ^{b}	12.68±0.11 ^{c}	13.78±0.17 ^{a}
Body weight (kg/cow)	-09.00±1.73 ^{b}	-21.00±0.57 ^{c}	03.00±0.57 ^{a}
BCS (1-5)	02.64±0.00 ^{b}	02.59±0.00 ^{c}	02.75±0.81 ^{a}
Glucose(mg/dl)	49.47±0.13 ^{b}	47.33±0.24 ^{c}	53.00±0.46 ^{a}
BUN(mg/dl)	14.90±0.17 ^{b}	16.60±0.22 ^{a}	13.10±0.05 ^{c}
Progesterone	0.21±0.03 ^{b}	0.16±0.66 ^{b}	0.73±0.11 ^{a}
LH	0.17±0.02 ^{b}	0.16±0.01 ^{b}	0.75±0.08 ^{a}

Values having different superscripts within same row are significantly different (P<0.05). DM: Dry matter, BCS: body condition score, BUN: blood urea nitrogen, LH: luteinizing hormone

4. Discussion

After parturition in dairy cows nutrient requirements increase whereas nutrient intake in term of dry matter intake reduces due to sudden change in animal physiology. To fulfill the nutrient requirements dairy cows mobilize their body reservoir (fats in adipose tissue) and loss rapid body weight and condition score ultimately lead to a state of loss in body condition score. Negative energy balance occurs due to lack of dietary intake and has a detrimental effect on animal production, reproduction and health by creating an imbalance in blood metabolites. This study suggests that the NRC recommended rations for dairy cows cannot meet the nutrient requirement early lactating cows in environment of Pakistan, extra energy is needed in diet 12% above NRC recommended levels, and attain better performance.

The results of the present study are in line with findings of [17] and [18], who reported that increasing dietary energy density in ration improved DM intake of dairy animals. In agreement to our results [13] reported a greater DMI (11.30 kg/day) for high energy diet (1.70Mcal NEL/kg) then 10.5 kg/day for low energy diet (1.58 Mcal NEL/kg). [19] Stated that DMI was slightly increased (4.84, 4.90, 4.93 kg/day) in Sahiwal heifers fed with different energy diet (80, 100 and 120% ME) in tropical environment. Reason for that might be due to the difference in animal physiological status, breed of animal, dietary ingredients and its nutritive composition, environmental changes and other managemental practices. In agreement to our results [22] found higher weight gain (694 kg) in Friesian cows fed with high energy concentrates and lower BWG (659kg) for low energy concentrate supported by such findings of [23]. Results of [6] in line with our results that gain in body weight was increased with 69.3% TDN then 59.7% TDN [24]. Recorded increased body weight by feeding of extra ME in feed to dairy cows. Similar results were reported by [25] and [26] observed loss in body weight and BCS at low energy feed. In early lactation adipose tissue uptake and utilization of glucose is reduced due to which body fats are mobilized in great extent.

High glucose level was recorded for high energy concentrate ration (3.46 mmol/L) compared to low energy concentrate (3.18 mmol/L) on day 37±3 postpartum [27, 28] Reported that plasma glucose concentration was (3.77 mmol/L) for cows receiving 8kg concentrate/day in their ration then cows fed with 4kg/ day concentrate in ration glucose level was 3.56 mmol/L postpartum [29, 30]. Reported that elevated level of insulin and glucose suggest positive energy balance in dairy cows after parturition [31, 32]. Reported decrease in glucose level after calving and remain low for few weeks then again increasing with increasing energy concentration in ration [33]. Observed higher glucose levels for grazing cows offered supplementary concentrate during lactation.

In line to our results [34] reported higher urea nitrogen concentration in blood and milk under negative energy dairy cows, which may be due to increase in body protein mobilization. Similar results were reported by [35, 36, 37]. that low dietary energy diet or high in protein to energy ratio diet increases BUN and milk urea nitrogen (MUN) due to reduced efficiency of rumen bacteria in utilizing free ammonia to synthesize protein and vice versa [38, 39]. suggested that due to NEB body fats and tissues were mobilized to compensate for the energy deficits and increases protein mobilizations ultimately increases BUN.

In agreement to our finding the results and results [40] of [41] that provision of an extra energy then the NRC recommended level enhanced the progesterone concentration in serum in comparison to low energy feed. Early lactating dairy cows which experience a state of NEB, suppress p4 concentration and P4 level decreased with restricted energy diet [42]. An agreement to our results [43] reported that plasma concentration of LH in dairy cows were 0.08 ng/ml for low energy diet while 0.09 ng/ml for high energy diet respectively postpartum. In agreement to current results similarly [44] found low level of LH (0.08 to 1.2 ng/ml) and (0.05 to 2 ng/ml) in early 30 days postpartum due to NEB in dairy cows.

5. Conclusion

It was concluded from the study that dairy cows receiving high energy diet (ME: 2.93 Kcal/kg DM) performed well than other diet in term of intake, body weight gain, body condition score, blood metabolites and reproductive hormones that are of most importance for recycling of dairy cow after parturition This study suggest that extra energy is needed in diet for early lactating cows above then NRC recommended levels, ME (12%) to attain good health with other important parameters studied in this study.

6. Acknowledgment

Author really acknowledged the laboratory staff of Microbiology and Histopathology department of Animal Health, The University of Agriculture Peshawar for their support in laboratory analysis. The Author pays special thanks to staff of University dairy farm Malakanadere Peshawar for their help throughout the experiment.

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