The Pharma Innovation Journal 2023; 12(3): 5835-5838

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 5835-5838

www.thepharmajournal.com
Received: 13-01-2023
Accepted: 16-02-2023

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Body condition score and levels of various energy and protein metabolic indicators in lactating Punganur cattle in two different seasons

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Abstract
The present study was conducted on Body Condition Score (BCS) and levels of various metabolic indicators in lactating Punganur cattle in two different seasons i.e., Autumn and Summer. The experiment was carried out with 24 Punganur cattle at Livestock Research Station, Palamaner. Serum samples were collected during summer (May and June) and autumn seasons (September and October) from six animals to estimate concentrations of Leptin, Ghrelin, NEFA, BHB and MUN in milk to study protein and energy metabolism in relation to BCS. In all animals mean BCS and mean body weights were significantly correlated ($p<0.01$). The body condition scoring was validated precisely by measuring body fat reserves using ultrasonography and BCS was highly correlated ($p<0.01$) with ultrasonographic fat reserves. The concentrations of Leptin & Ghrelin did not differ significantly ($p>0.05$) between the two seasons, where the BCS and serum leptin concentration of animals showed significant positive correlation (r = 0.744). MUN levels during autumn and summer seasons were 7.32±1.0 and 4.98±0.68 (mg/dl) respectively. BHB and MUN values were significantly ($p<0.05$) higher during summer whereas NEFA levels did not differ significantly. NEFA and BHB values were found to be significantly correlated (r = 0.938) during both the seasons. Monitoring concentrations of metabolic indicators are essential for early identification of metabolic disturbances.

Keywords: BCS (body condition score), leptin, Ghrelin, non-esterified fatty acids, beta hydroxy butyrate, MUN (milk urea nitrogen)

1. Introduction

Body condition scores could provide an acceptable and useful estimate of the proportion of fat in the live animal and that the level of prediction was superior to that afforded by live weight (Russel *et al.*, 1969) [19]. BCS is widely used in livestock production and contributes to the decisions regarding different management practices during production cycle such as selection of the mating period or when it is necessary to use energy supplementation during lactation (Short *et al.*, 1996) [20]. High correlation between ultrasonic measurements and condition score, suggesting that condition score reflects the actual quantity of subcutaneous fat (Zulu *et al.*, 2001) [25]. Reduced synthesis of leptin in white adipose tissue is largely responsible for the lower concentration of plasma leptin in early lactating dairy cows and this reduction could benefit early lactating dairy cows by promoting a faster increase in feed intake and by diverting energy from non-vital functions such as reproduction (Block *et al.*, 2001) [4]. BCS in late pregnant cows explained 37% of the variation in plasma leptin showing that plasma leptin was positively correlated with BCS in cows during lactation (Ehrhardt *et al.*, 2000) [6]. Circulating ghrelin concentrations increase during fasting or negative energy balance in dairy cows and exogenous administration of ghrelin stimulates feed intake in cattle and the average pulse concentrations were greater ($p<0.001$) for fasting steers compared with well-fed steers (Wertz-Lutz *et al.*, 2006) [22]. Ghrelin contributes to adaptations that occur during long-term fasting, but no association between endogenous ghrelin release and growth hormone secretion was found in mature dairy cattle fed to nutrient requirements (Miura *et al.*, 2004) [14]. The metabolic profile indicators are of great importance for early identification of energy metabolism disturbances in cows and most reliable indicators of the cow's energy status are β-hydroxybutyrate (BHB) and glucose concentrations and are in high correlation with blood NEFA concentrations and BCS during the peripartal period (Prodanović *et al.*, 2010) [18]. Energy deficiency during lactation causes fat mobilization from the body deposits, increased concentration of Non Esterified Fatty Acids (NEFA) in the blood and increased milk fat synthesis in the mammary gland (Pechová and Pavlata, 2005) [16].

Circulating metabolites (NEFA and BHBA) are commonly used indices of negative energy balance or ketosis in transition animals (Herdt, 2000) [10].

2. Materials and Methods

The study was carried out with Punganur cattle at Livestock Research Station, Palamaner. The research work was planned to study the body condition scoring in 24 punganur lactating animals and among 24 animals, in six animals body condition scoring was validated by measuring body fat reserves using ultrasonography and Serum samples were collected during summer and autumn from six animals to study protein and energy metabolic indicators like MUN, Leptin, Ghrelin, NEFA and BHB during both seasons. The laboratory analysis was carried out in Department of Animal Nutrition, Department of Veterinary Biochemistry and Teaching Veterinary Clinical Complex, Tirupati. The chart for condition scoring in a 1 to 5 scale using 0.5 increments, functioning as a 9-point scale was used (Anitha *et al.*, 2005) [2]. A score of 1 indicates emaciated, 2 indicate thin, 3 indicate average, 4 indicate fat and 5 indicates obese condition. Eight skeletal checkpoints were examined and merits within each area were used to indicate the body conditions which were 1. Tail head to pin bones. 2. Spinous processes of the lumbar vertebrae. 3. Depression between the spinous and transverse processes. 4. Transverse processes of lumbar vertebrae. 5. Point between 12th and 13th ribs. 6. Sacral crest. 7. Depression between sacral crest and hooks and 8. Depression between hooks and pins. After each check point was observed by vision and palpation, the scores were recorded and average BCS was assigned to the selected animals in herd. The BCS and ultrasonographic measurements were recorded independently for the same animal on the same day at five body locations. The five locations were the 1. Area between the tail head and pin bones, 2. Lumbar area, 3.12th and 13th ribs, 4. Area between the sacral crest and tubercoxae and 5. Midway between hooks and pins above the greater trochanter of the femur and measurements were obtained by freezing the image on the screen of the ultrasound machine. Blood sample collection: Blood samples were collected from six lactating animals in two seasons during the summer and autumn in the morning before feeding. Approximately 6 ml of blood was collected into vacutainers by jugular vein puncture. After one hour, the serum was separated and centrifuged at 3000 rpm for 5 minutes to get clear serum. The serum was stored at -20°C for the hormonal estimation. All the selected animals were in good health condition at time of study. The body weights of the animals were taken before feeding on the day of collection of blood samples by using the mechanical weigh bridge. Serum leptin and ghrelin concentrations were determined using a leptin (Standard curve range: 0.05ng/ml-10ng/ml) and ghrelin (Standard curve rang: 30ng/l-9000ng/l) sandwich ELISA kit (Bioassay technology laboratory, Bovine specific) as per the manufacturer's instructions. To estimate energy metabolic indicators NEFA and BHB, NEFA calorimetric assay and BHB competitive ELISA kit (ELK biotechnology) are used respectively and protein metabolism is estimated by studying MUN (Milk Urea Nitrogen) where the milk serum samples were used for MUN estimation by Rahmatullah and Boyde method.

2.1 Statistical analysis: The analysis was run through software (version 20; SPSS, 2015) by applying two-way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test.

3. Results

Body condition scores were assigned in 24 lactating animals in a 1 to 5 scale using 0.5 increments by examining the 8 skeletal checkpoints on each animal. The mean scores at the skeletal points 1, 2, 3, 4, 5, 6, 7 and 8 were 2.97, 2.75, 2.75, 2.83, 2.82, 2.75, 2.65 and 2.82 respectively. The mean BCS of the lactating animals was 2.79 and the mean body weights of all lactating animals was 147.04 kg. The mean BCS and mean body weights were significantly correlated ($p<0.01$) in case of lactating animals. The mean±SE values of fat thickness (mm) at the check points between tail head and pins, lumbar area, between 12th and 13th ribs, between sacral crest and hooks and between hooks and pins were 3.58±0.20, 3.0±0.22, 2.91±0.41, 3.33±0.33 and 3.08±0.45 respectively. Correlation between BCS and body fat reserves showed that BCS was highly correlated ($p<0.01$) with ultrasonographic fat reserves. Serum samples were collected during summer and autumn from six animals to study protein and energy metabolic indicators like MUN, Leptin, Ghrelin, NEFA and BHB. The serum leptin concentration (ng/ml) during the autumn and summer seasons was 0.95±0.16 and 0.71±0.18 respectively. The concentrations of leptin did not differ significantly ($p>0.05$) between the two seasons. The BCS and serum leptin concentration of animals showed significant positive correlation (r = 0.744) (Table1).

Table 1: Correlation between BCS and serum Leptin concentrations of animals

	BCS	**Serum leptin oncentration**
BCS	---	0.744**
Serum leptin concentration	0.744**	---

** Significant ($p<0.01$)

The serum ghrelin concentration (ng/ml) during the autumn and summer seasons were 0.88±0.20 and 1.14±0.33 respectively where the concentrations of ghrelin did not differ significantly ($p>0.05$) between the two seasons. The levels of all the above indicators are mentioned in table 2.

Table 2: The levels of all the above indicators in two different seasons

Hormone	**Leptin** (ng/ml)	**Ghrelin** (ng/ml)
Autumn	0.95±0.16	0.88±0.20
Summer	0.71±0.18	1.14±0.33

The concentrations of milk urea nitrogen (MUN), Nonesterified Fatty Acids (NEFA) and Betahydroxybutyrate (BHB) in milch animals under two different seasons were presented in Table 3. Milk urea nitrogen levels were 7.32±1.0 and 4.98±0.68 (mg/dl) during summer and autumn season respectively and showed significantly ($p<0.05$) higher values during summer as compared to autumn season. NEFA levels were 0.49±0.03 and 0.41±0.03 (mmol/L) during summer and autumn season respectively and did not differ significantly ($p>0.05$). BHB levels were recorded as 1.25±0.11 and 0.92±0.05 (mmol/L) during summer and autumn season respectively which showed significantly ($p<0.05$) higher values during summer. The NEFA and BHB values were found to be significantly correlated (r = 0.938) during both the seasons in case of milch animals.

Table 3: Concentration of Milk Urea Nitrogen, NEFA and BHB in milch animals in two seasons

Metabolite	Summer season	Autumn season
MUN	7.32±1.0[a]	4.98±0.68[b]
NEFA	0.49±0.03[a]	0.41±0.03[a]
BHB	1.25±0.11[a]	0.92±0.05[b]

Mean±SE with different superscripts differ significantly within the rows (*p*<0.05)

4. Discussion

The BCS is determined by vision and palpation of the skeletal check points which was in tune with (Ferguson *et al.*, 1994) [8] who examined the body locations by appearance and palpation, whereas (Edmonson *et al.*, 1989) [5] evaluated the body locations only visually. The skeletal check points were identified by spotting out the anatomical features which enabled to assess the fat reserves easily. The mean scores at the skeletal points 1, 2, 3, 4, 5, 6, 7 and 8 were 2.97, 2.75, 2.75, 2.83, 2.82, 2.75, 2.65 and 2.82 respectively and the mean BCS of the lactating animals was 2.79 and the mean body weights of all lactating animals was 147.04 kg. The mean BCS and mean body weights were significantly correlated (*p*<0.01) in case of lactating animals which was in accordance with (Berry *et al.*, 2003) [3]. Thus, by using BCS one could assess the weight gain or loss, avoid the negative effects of under or over feeding cattle and also select healthy animals while purchasing.

Ultrasonography is relatively a unique managing modality with characteristic features of non-invasiveness and repeatability, free from radiation hazards and allows precise location of instrument for collection of material from desired site as reported by (Franz *et al.*, 2009) [9]. In present study the mean±SE values of fat thickness (mm) at the check points between tail head and pins, lumbar area, between 12th and 13th ribs, between sacral crest and hooks, between hooks and pins were 3.58±0.20, 3.0±0.22, 2.91±0.41, 3.33±0.33 and 3.08±0.45 respectively. As the BCS increased the amount of fat reserves increased significantly (*p*<0.01) indicating that BCS adequately reflected in the amount of actual fat reserves which was in accordance with (Zulu *et al.*, 2001) [25] who reported significant correlations between BCS and ultrasonographic measurements of subcutaneous fat in dairy cows. During undernutrition leptin plays an important role in the adaptation of animal and rapid decrease in plasma leptin in underfed animals could be an acute signal to stimulate re-feeding behaviour (Ahima *et al*., 1996) [1]. The serum leptin concentration (ng/ml) during the autumn and summer seasons was 0.95±0.16 and 0.71±0.18 respectively. The concentrations of leptin did not differ significantly (*p*>0.05) between the two seasons. However, they were numerically lower in all animals during summer compared to autumn which is in agreement with (Liefers *et al.*, 2003) [12] as heat load animals exhibit reduced feed intake in order to reduce metabolic heat according to (West, 2003) [23]. In present study BCS and serum leptin concentration in all 6 animals showed significant positive correlation (r = 0.744, *p*<0.01) which is in agreement with (Vargova *et al*., 2015) [21] and multiparous cows with higher BCS had higher leptin concentration compared to primiparous cows as reported by (Miekle *et al.*, 2004) [13]. So optimum body condition scores are to be maintained in dairy animals in order to maintain the normal concentrations of leptin so that animal will not undergo negative energy balance. The concentrations of ghrelin increase during fasting or negative energy balance in dairy cows, and with exogenous administration of ghrelin stimulates feed intake as reported by (Wertz-Lutz *et al*., 2006) [22]. The serum ghrelin concentration (ng/ml) during the autumn and summer seasons were 0.88±0.20 and 1.14±0.33 respectively where the concentrations of ghrelin did not differ significantly (*p*>0.05) between the two seasons and the mean ghrelin concentrations were numerically higher during summer compared to autumn. As the availability of feed resources were less during the summer, adaptations to negative energy balance in dairy cattle appear to include meal-associated increase in ghrelin and long - term energy restriction increased plasma ghrelin concentration even in small ruminants as reported by (Kurose *et al*., 2005) [11]. Milk urea nitrogen (MUN), a fraction of milk protein that is derived from blood urea nitrogen (BUN), may be one of the useful tools that may help monitoring of any change required in the feeding and management of a herd (Peterson *et al.*, 2004) [17]. In the present study milk urea nitrogen levels showed significantly (*p*<0.05) higher values during summer as compared to autumn which is in agreement with (Fatehi *et al*., 2012) [7] who reported that milk urea nitrogen was at its lowest level during winter months (13 mg/dL), reached a maximum in summer (18.8 mg/dL) and progressively diminished to the autumn revealing that milk urea nitrogen concentration was positively correlated with monthly temperature mean and may be a reason for the lower reproductive performance during the summer months. In present study NEFA levels were 0.49±0.03 and 0.411±0.03 (mmol/L) during summer and autumn season respectively and did not differ significantly (*p*>0.05) between seasons which is in accordance with (Wheelock, 2010) [24]. BHB levels were 1.25±0.11 and 0.92±0.05 (mmol/L) during summer and autumn season respectively which showed significantly (*p*<0.05) higher values during summer. This is in accordance with (Moreria *et al*., 2015) [15] who stated that the concentration of BHB increased up to five days postpartum and remained high subsequently, while NEFA concentrations were declined which may be due to fact that during study the test group of animals were in early stages of lactation. The NEFA and BHB values were found to be significantly correlated (r = 0.938) during both the seasons which were in accordance with (Prodanović *et al*., 2010) [18] who stated that the concentrations of BHB and glucose were in high correlation with blood NEFA concentrations and BCS.

5. Conclusion

BCS can be used as effective tool to determine the animal fitness. The concentrations of leptin and ghrelin were at optimum levels in animals with good body condition scores. Estimating the concentrations of metabolic indicators like NEFA, BHB and MUN revealed that during stress conditions the ketone bodies and protein metabolite levels increased which may be due to decreased feed intake and altered nutrient metabolism. Thus, monitoring of these metabolic indicators is of great importance in maintenance of heard health especially during transition period to prevent the animal from negative energy balance, which can be done by following the appropriate feeding practices to maintain the optimum body condition scores that prevent the metabolic disorders like ketosis, milk fever.

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