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Research Article

PERIPHERAL STUDY ON PLASMA LEPTIN AND GHRELIN IN RELATION TO BODY WEIGHT AND BIOCHEMICAL PARAMETERS IN GROWING BUFFALOES

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ABSTRACT

The study was conducted to assess the levels of leptin, ghrelin and biochemical parameters in plasma during growth, pre-pubertal, pubertal and post-pubertal stages in buffaloes and to correlate them with body growth parameters. Thirty buffaloes selected at random from the animal herd of buffalo farm, LUVAS, Hisar were divided into five groups *viz*. buffalo calves of age 6-9 months (group 1), 10-14 months (group 2), 15-20 months (group 3), pubertal buffaloes (group 4), post-pubertal period 1 to 4 months (group 5). Blood samples were collected in morning before offering feed at specific interval. Plasma hormones *viz*. leptin, ghrelin and blood biochemical parameters (Glucose, Triglycerides, Magnesium, Calcium, Phosphorus) and electrolytes were estimated. Body weight and other growth parameters (abdominal girth, body height, body length, and heart girth) were also recorded at specific intervals of experimental period. The results revealed a significant increasing trend of mean body weight and other growth parameters at different intervals within the groups. A significant difference in leptin and ghrelin concentration was observed in group 4 (pubertal) and group 5 (post pubertal) animals ($p<0.05$). A negative correlation was observed between leptin, ghrelin and body weight, whereas leptin did not show correlation with triglycerides and glucose. Ghrelin showed a positive correlation with body weight and negative correlation with leptin hormone whereas, no correlation was observed with triglycerides and glucose. It can be suggested that leptin and ghrelin having a counteracting effect and may play a crucial role in the body growth.

Keywords: Biochemical, Buffaloes, Electrolytes, Ghrelin, Growth, Leptin

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Body growth parameters and body weight are considered to be important criteria to attain puberty in animals. Body weight is controlled by anorexigenic and orexigenic factors, more often by different peptidic hormones, such as; leptin, ghrelin and adiponectin. The adipose derived hormone leptin, anorexigenic hormone and ghrelin (hunger hormone) and others like Nefstatin-1 act as indicators of energy status influencing the carbohydrate, lipid and protein metabolism. Leptin plays a major role in regulation of energy balance by reducing feed intake and increasing energy expenditure mediated through central hypothalamus (Khaki *et al.*, 2014). Ghrelin has been found to exert a plethora of physiological effects. It induces appetite, stimulates the gastric acid secretion and motility, promotes the carbohydrate metabolism as a source of fuel, inhibits lipid oxidation, promotes lipogenesis and improves cardiac performance. It has the ability to modulate gonadotropin secretion, to influence the onset of puberty and to directly regulate gonadal physiology.

The knowledge of the physiological role of leptin and ghrelin in domestic animal's peripheral metabolism is limited as compared to rodents and human studies. Thus, understanding the basic mechanisms that regulate adiposity, feed intake and energy metabolism in livestock may lead to new approaches which can be helpful in further improving animal performance and health. Keeping all the points in view, the present study was conducted to know the plasma levels of leptin, ghrelin and biochemical parametersin relation to growth in buffaloes.

MATERIALS AND METHODS

The present study was conducted from September, 2019 to Feb., 2020 on animals selected from the buffalo farm, Livestock Production and Management, LalaLajpat Rai University of Veterinary and Animal Sciences (LUVAS). Hisar city is situated in semi-arid region and climatic conditions are sub-tropical in nature. Geographically, Hisar is situated at 29° 10' N latitude, 75° 40' E longitude and 215.2 meters altitude. Prior approval was taken to conduct the present investigation by the institutional animal ethical committee (IAEC).

Animal selection and feeding

Thirty buffaloes selected at random from the animal herd of buffalo farm, LUVAS, Hisar were divided into five groups having six animals in each group *viz*. buffalo calves of age 6-9 months (group 1), 10-14 months age (group 2), 15-20 months age (group 3), pubertal buffaloes (group 4), post-pubertal period of 1 to 4 months buffaloes (group 5). Animals selected for the study were free from any anatomical, physiological and infectious disorders. During the experimental period, the animals were given green fodder and concentrate mixture so as to meet their protein and energy need for growth as per ICAR, 2013

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feeding standards. The quantity of different feed given to each group was adjusted at fortnightly interval so that the overall DCP and TDN requirements of calves were met according to the change in body weight. Animals were allowed ad lib fresh drinking water throughout the experimental period.

Body growth measurements and blood sampling

Body weight and body growth parameters (abdominal girth, body height, body length, and heart girth) (cms) at different intervals of experimental period were measured in the morning before offering feed (Table 1). Blood samples were collectedin 15 ml heparinized polypropylene tubesin morningat same specific interval mentioned in Table 1 from all animals by means of jugular vein puncture using 18-guage stainless steel needle (Dispovan, India). The plasma was separated by centrifugation of blood at 3000 rpm for 15-20 min at 4 ºC within an hour after sampling and collected inthe storage vials of 2 ml capacity (Tarson, India) and stored at -20 ºC in the deep freezer.

Biochemical analysis

Leptin and ghrelin hormonal analysis was done by Enzyme-Linked Immunosorbent Assay (Bovine ELISA Quantization kits, Bioassay lab). Plasma biochemical parameters (glucose, triglycerides, magnesium, cholesterol, calcium, phosphorus, chloride) were analysed by semi auto analyser using kits supplied by Erba, Mammheim. Electrolytes (sodium, potassium) were estimated by flame photometry, systronic, following standard protocol. The data were analyzed by SPSS software.

RESULTS AND DISCUSSION

Body growth parameters: The results revealed an increasing trend in body weight at different stages in each group throughout the experiment (Table 1). The mean body weight of all the experimental animals at different intervals was observed to be significantly different in group 2, group 3 and group 5 ($p<0.05$). The results revealed an increasing trend with advancement of age in body growth parameters related to animal health and performance (body length, body height, hearth girth) in each group throughout the experimental period.

Body weight and other body size criteria such as wither height, body condition score, body length, height, heart girth and pelvic area are used to aid growth definitions as they are more often related to animal performance and health than body weight alone (Vahora *et al.*, 2012). Anorexigenic and orexigenic factors, different peptidic hormones, such as; leptin, ghrelin and adiponectin are considered to be as important factors regulating body weight. It has been reported that buffalo heifers attain puberty when reach about 55-60% of their adult body weight. Nutritional, environmental are other contributing factors towards pubertal age ultimately affect efficiency of animals (Castaneda *et al.*, 2010; Ahmadi *et al.*, 2016). Leptin is involved in a series of biological functions including regulation of feed intake, secretion of growth hormone, body weight and gastrointestinal motility (Vrieseand Delporte, 2008). Plasma leptin levels are highly correlated with body fatness mass in bovines, ovines, rodents and equines, although diet modulation, energy consumption and feeding behavior play an important role in regulating body fatmass. Reports on role of leptin in metabolic control of onset of puberty, feed intake and energy homeostasis are available in cattle, human, rat, mice, pig, and fish (Anwar *et al.*, 2014; Zuure *et al.*, 2013).

Plasma hormones and biochemical parameters: The statistical analysis of data revealed a significant difference in leptin and ghrelin concentration between groups of different physiological stages ($p<0.05$) (Table 2). In a study on gilts, the author observed an age-related increase in serum leptin concentration during 90, 150 and 210 days of their life (Qian *et al.*, 1999). Similar results were observed in present study in group 1 and group 2 but reduced level of leptin were observed in pubertal and post pubertal animals. In an experiment conducted to describe the plasma leptin variation in dairy heifers shortly after weaning until puberty, at different days around puberty, in early pubertal and late pubertal groups, the plasma concentration of leptin was nearly constant around puberty in the early pubertal heifers, whereas an increased level was observed in late pubertal heifers (Block *et al.*, 2003). In present study also, the plasma leptin was increased in group 3 significantly but it was non-significant in group 4. It was suggested that plasma leptin does not determine the onset of puberty as body weight, mean plasma leptin and leptin profile of both groups remained to be similar. But contradictory to this, a hypothesis was given by Foster and Nagatani (1999) that leptin could be a primary signal triggering puberty. Therefore, an age dependent rise in plasma leptin cannot be considered as development event triggering the onset of puberty in ruminants (Cheung *et al.*, 2001).

Sodium and potassium concentration of all the experimental animals at specific intervals was observed to be significantly different between the groups and a non significant difference in chlorideconcentration was observed between groups of different physiological stages ($p<0.05$).

Pasha *et al.* (2012) recorded a significant difference in sodium and potassium concentration ($p>0.05$) among

Table 1

Body growth parameters (cms) and body weight (pounds) at different intervals (Mean±SE)

PARAMETERS/ Groups	Group 1(n=6)			Group 2 (n=6)			Group 3 (n=6)				Group 4 (n=6)	Group 5 (Post Puberty) n=6		
	180 Days	225 Days	270 Days	315 Days	360 Days	405 Days	450 Days	495 Days	540 Days	585 Days	within one week of Puberty	30 days pp	75 days pp	120 days pp
Abdominal girth (cm)	45.83^{a} ±2.03	53.66^{b} ±1.40	55.83^{b} ±1.37	54.16 ±1.04	56.5 ±1.28	58.16 ±1.51	56.83^{a} ±1.83	59.66^{ac} ±1.45	68.00^{bce} ±3.51	72.00^{bde} ±3.10	79.83 ±0.47	81.66^{a} ±1.05	84.6^{b} ±0.61	86.3^{b} ±0.66
Body Height (cm)	37.16^{a} ±1.27	40.16^{a} ±0.79	41.66^{b} ±0.84	40.16^{a} ±0.60	42.83^{b} ±0.79	43.66^{b} ±0.80	40.33^{a} ±1.054	44.16^{ac} ±1.16	47.33^{ac} ±2.20	53.00^{bd} ±3.44	53.66 ±0.80	53.83 ±0.65	55.00 ±0.81	56.00 ±0.93
Body Length (cm)	30.16^{a} ±0.47	34.00^{b} ±1.12	35.50^{b} ±1.20	32.00^{a} ±1.03	35.16^{b} ±0.54	37.16^{b} ±0.47	36.66^{a} ±1.11	40.33^{a} ±1.54	43.16^{b} ±2.28	45.83^{b} ±2.21	49.83 ±1.44	51.66 ±1.08	52.33 ±1.05	53.50 ±0.99
Heart Girth (cm)	42.00 ±2.04	45.83 ±2.50	46.33 ±1.56	49.83^{a} ±0.47	53.00^{b} ±0.93	53.16^{b} ±1.01	52.66^{a} ±2.10	55.00^{ac} ±1.77	63.16^{bc} ±3.59	67.16^{bd} ±3.72	76.50 ±0.56	76.83^{a} ±1.79	77.66^{a} ±1.60	82.33^{b} ±0.71
Body Weight (pounds)	179.0 ±17.47	244.68 ±33.35	264.54 ±15.94	264.35^{a} ±5.22	329.55^{b} ±11.32	350.52^{b} ±12.38	344.59^{a} ±34.19	410.42^{ac} ±32.55	601.00^{ace} ±109.8	718.14^{bdf} ±118.59	973.36 ±37.41	1023.14^{a} ±61.55	1057.06^{a} ±55.79	1208.33^{b} ±21.41

a,b,c ,d,,e,fMean values bearing different superscript within a row varies significantly ($p<0.05$)

Table 2

Plasma hormones and biochemical parameters in different groups (Mean±SD)

Groups	Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)	Group 4 (n=6)	Group 5 (n=6)	P-value
Leptin (ng/ml)	11.73±3.40^{a}	13.09±3.81^{ac}	11.87±2.88^{acx}	4.29±0.72^{bdye}	8.62±2.74^{bdyf}	0.0123
Ghrelin (ng/l)	677.85±67.79^{a}	495.68±67.42^{bc}	577.56±65.89^{bdx}	897.94±38.97^{bdye}	630.59±35.19^{adyf}	0
Glucose (mg/dl)	69.94±17.10	67.31±11.54	73.94±13.02	68.66±8.69	68.81±11.25	0.545
Triglycerides (mg/dl)	18.08±3.83	16.08±1.71	17.60±2.68	17.33±1.69	16.64±2.13	0.1748
Cholesterol (mg/dl)	24.82±5.92^{a}	19.04±2.85^{b}	18.69±2.95^{b}	19.32±2.45^{b}	17.43±2.32^{b}	0
Magnesium (mmol/l)	2.58±0.04	2.6±0.14	2.61±0.04	2.63±0.15	2.55±0.17	0.9491
Phosphorus (mg/dl)	5.85±0.85	5.37±1.09	5.64±0.62	5.25±0.67	5.34±0.48	0.1951
Calcium(mg/dl)	10.33±0.50^{a}	9.83±0.45^{bc}	9.65±0.27^{bcx}	9.33±0.19^{bdx}	9.21±0.30^{bdy}	0
Sodium (mmol/l)	137.91±12.72^{a}	151.66±14.21^{bc}	137.905±11.27^{bd}	145.08±6.60^{ac}	135.79±13.19^{ad}	0.0014
Potassium (mmol/l)	4.09±0.93^{a}	4.48±1.22^{ac}	4.33±1.08^{acx}	3.21±0.21^{adx}	3.17±0.40^{bdy}	0.0001
Chloride (mmol/l)	95.95±1.71	93.62±7.44^{c}	99.34±6.60^{dx}	92.4±7.3^{cy}	93.95±1.46^{cy}	0.2207

a,b,c ,d,x,y,e,fMean values bearing different superscript within a row varies significantly ($p<0.05$)

different physiological stages of buffaloes in rice zone of Punjab province. They also recorded a non-significant difference in phosphorus concentration and a significant difference in calcium concentration ($p>0.05$) among different physiological stages which suggests that physiological stage of buffalo has no or little effect on plasma phosphorus.

Correlation of leptin and ghrelin withbody weight and biochemical parameters

Pearson correlation value of leptin with ghrelin, body weight, glucose, triglycerides and cholesterol was calculated. The R- value for ghrelin was (-) 0.9073 and for body weight was (-) 0.8089. The results revealed that body weight and ghrelin are negatively correlated with leptin whereas no correlation of leptin and ghrelin was observed withglucose, triglycerides and cholesterol.

Pearson correlation value of ghrelin with leptin, body weight, glucose, triglycerides and cholesterol was calculated. The results revealed that ghrelinis positively correlated with body weight (R value 0.5163) and negatively correlated with leptin.

Growth rates are dependent on the net energy available to the animal after the maintenance requirements are met (NRC, 2001). In the present study negative correlation has been observed between leptin and body weight. The lipostat theory is the process by which animals gets signal to inhibit the appetite and consume its own energy stores whenever the body senses pre set limits. Insulin stimulates glucose, free fatty acid and amino acid uptake by tissues and tissue anabolism by directly acting on adipocytes by increasing leptin gene expression, perhaps due to increased glucose transport and metabolism (Margetic et al., 2002). The physiological impact of leptin in nutrient stimulation and the mechanism underlying the role of leptin in insulin secretion and glucose regulation

still needs to be studied in more species.

Maximum increase in ghrelin has been observed in group 4 animals (pubertal group). Ghrelin is known to be the peripheral inhibitor of insulin and leptin, as ghrelin is active even with peripheral administration in contrast to other orexigenic peptide which are only active when injected into brain. It also reduces glucose stimulated insulin secretion. The metabolic changes observed during different physiological stages in animals indicate their status to meet energy demand of their body (Fiore et al., 2018). The events suggest that fatness and or leptin can play role as permissive regulator of puberty independently or in combination (Chilliard et al., 2005). Singh et al. (2018) studied relation between residual feed intake and blood biochemical parameters in growing buffalo calves and reported a negative but non-significant correlation between plasma glucose and total protein with residual feed intake. The concentration of IGF-1 and leptin in blood plasma were higher ($P<0.05$) in low RFI group and were negatively correlated with residual feed intake. The results observed suggest that leptin and ghrelin have counteracting effects as orexigenic and anorexigenic by stimulating hypothalamic neurons resulting in more or less opposing effects on energy balance. Leptin is independent of body weight in terms of attaining puberty in animals. Diet modulator or plane of nutrition, feeding behaviour and other social factors are also contributing factors to puberty.

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REFERENCES

Ahmadi, M., Pacala, N., Bencsik, I., Dronca, D., Stef, L., Nichita, I. and Scurtu, M. (2016). The importance of leptin in animal science. Scientific Papers. *Anim. Sci. Biotechnol.* **49(1)**: 134-139.

Anwar, G.M., Yamamah, G., Ibrahima, A., El-Lebedy, D., Farid, T.M. and Mahmoud, R. (2014). Nesfatin-1 in childhood and adolescent obesity and its association with food intake, body composition and insulin resistance. *Regulatory Peptides.* **188**: 21–24.

Block S.S., Smith, J.M., Enhardt, R.A., Diaz, M.C., Rhoads, R.P., Van Amburgh, M.E. and Boisclai, Y.R. (2003). Nutritional and developmental regulation of plasma Leptin in dairy cattle. *J. Dairy Sci.* **86**: 3206–3214.

Castaneda, T.R., Tong, J., Datta, R., Culler, M. and Tschop, M.H. (2010). Ghrelin in the regulation of body weight and metabolism. *Frontiers in Neuroendocrinol.* **31(1)**: 44–60.

Cheung, C.C., Thornton, J.E., Nurani, S.D., Clifton, D.K. and Steiner, R.A. (2001). A reassessment of Leptin's role in triggering the onset of puberty in the rat and mouse. *Neuroendocrinology*. **74**: 12-21.

Chilliard, Y., Delavaud, C. and Bonnet, M. (2005). Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. *Domestic Anim. Endocrinol.* **29**: 3-22.

Fiore E., Arfuso, F., Gianesella, M., Vecchio, D., Morgante, M., Mazzotta, E., Badon, T., Rossi, P., Bedin, S. and Piccione, G. (2018). Metabolic and hormonal adaptation in *Bubalus bubalis* around calving and early lactation. *PloS one.* **13(4)**: e0193803.

Foster, D.L. and Nagatani, S. (1999). Physiological perspectives on leptin as a regulator of reproduction: role in timing puberty. *Biol. Repro.* **60(2)**: 205-215.

ICAR (2013). Nutrient composition of Indian feeds and fodders, (1st Edn,) Indian Council of Agricultural Research and National Institute of Animal Nutrition and Physiology. New Delhi.

Khaki, A., Batavani, R., Najafi, G., Tahmasbian, H., Belbasi, A. and Mokarizadeh, A. (2014). Effect of leptin on in vitro nuclear maturation and apoptosis of buffalo (*Bubalus bubalis*) oocyte. *Int. J. Fert. Steril.* **8(1)**: 43-51.

Margetic, S., Gazzola, C. Pegg, G.G. and Hill, R.A. (2002). Leptin: a review of its peripheral actions and interactions. *Int. J. Obes Relat. Metab. Disord.* **26**: 1407-1433.

NRC (2001). Nutrient Requirements of Dairy Cattle. 7th Revised Edition. National Academy Press, Washington, DC.

Pasha, T.N., Khan, M.Z., Farooq, U., Ditta, A.Y., Ilyas, M. and Ahmad, H. (2012). Macro-minerals status of buffaloes in rice zone of Punjab province. *J. Ani. Plant Sci.* **22(3)**: 319-323.

Qian, H., Barb, C.R., Compton, M.M., Hausman, G.J., Azain, M.J., Kraeling, R.R. and Baile, C.A. (1999). Leptin mRNA expression and serum leptin concentrations as influenced by age, weight, andestradiol in Pigs. *Domes. Anim. Endocrinol.* **16**: 135-143.

Singh, V., Madan, J., Sindhu, S., Kumar, Ravi and Paul, S.S. (2018). Residual feed intake and its relationship with blood biochemicals in growing buffalo calves. *Haryana Vet.* **57(1)**: 38-41.

Vahora, S.G., Parnerkar, S. and Kore, K.B. (2012). Effect of feeding bypass nutrients to growing buffalo heifers under field conditions. *Liv. Res. Rural Dev.* **24(2)**: http://www.lrrd.org/lrrd24/2/vaho24024.htm.

Vriese, D.C. and Delporte, C. (2008). Ghrelin: a new peptide regulating growth hormone release and food intake. *Int. J. of Biochem. Cell Biol.* **40**: 1420–1424.

Zuure, W.A., Roberts, A.L., Quennell, J.H. and Anderson, G.M. (2013). Leptin signaling in GABA neurons, but not glutamate neurons, is required for reproductive function. *J. Neurosci.* **33**: 17874-17883.