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RESEARCH ON THE METABOLIC PROFILE OF BUFFALO COWS FROM THE ROMANIAN BUFFALOES BREED, PRE AND POST-PARTUM

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Abstract

The present study aimed to monitor the changes in metabolic profile during the pre- and post-partum period of high milk producing buffalo cows and their relationship with herd management. The transition period is defined as the period from 3 weeks before calving to 3 weeks after calving. Among the factors that influence the metabolic profile of buffalo cows, the following were highlighted: nutrition, reproduction and climatic factors. This study was carried out on two batches of 10 buffaloes in different stages of the gestation period. The buffaloes benefited from the same fodder ration before going out to graze. After going out to the pasture, the animals taken in the study were fed only with grass from the natural pasture. Ante partum and post-partum blood samples were collected. The samples were analyzed biochemically. The results draw attention of the metabolic profile of the blood in evaluating the nutritional status of buffalo cows and ensuring good health in very demanding physiological conditions, in addition, they provide some indications that buffaloes are more resistant to metabolic disorders in the post-partum period.

Key words*: buffalo cows, metabolic profile, transition period.*

INTRODUCTION

In Romania, the buffalo entered with the invasion of the Huns and Avars in the Carpatho-Danubian area. It found the good pedo-climatic conditions and so, in our country, has developed a buffalo population which had its own evolutionary path as a result of reproductive isolation (Vidu et al., 2008).

Romania has the major advantage of the existence of a research station in the field of buffalo breeding, which has an extremely valuable breeding nucleus. Increasing the economic efficiency of buffalo production and developing a breeding program are keys to actively conserving of this genetic structure. The buffalo is a species with remarkable quality, of which we can remember: high percentage of milk fat, meat with exceptional taste qualities, resistance to diseases and heavy environmental conditions, good valorization of poor quality feeds.

Immediately before parturition as well as during the first stage of lactation, increased mammary gland activity results in energy deficiency and increased lipomobilization from body reserves. Many authors investigated the buffalo metabolic response to lactation since buffaloes show a different pattern compared to other ruminants as demonstrated by the low incidence of metabolic disorders.

The centre of animal physiology is the homeostasis of the glucose, which involves primarily the somatotropine, insulin, glucagon and glucocorticoids with thyroid hormones being usually decreased after calving, with the aim to reduce tissue metabolism in order to have a higher nutrient availability for mammary gland metabolism.

The transition period is defined as the period between 3 week before to 3 week after parturition, and it is critically important to the health and profitability of dairy cows as well as in buffaloes. This phase is characterized by major physiological, nutritional, metabolic, and immunological changes. Buffaloes adjust their metabolism to deal with the considerable increase of energy and nutrient requirements needed for milk production which makes them susceptible to negative energy balance (NEB).

The metabolic adaptation to NEB requires interactions with different energy resources, and its failure may occur in various tissues such as the liver, adipose tissue, and others. Metabolic disorders in clinical or subclinical form are manifestations of the animal's inability to manage the greater metabolic demands. The metabolic response to lactation of buffaloes shows a different pattern compared with other ruminants, as demonstrated by the low incidence of metabolic disorders.

Unlike lipid metabolism, protein metabolism is not markedly influenced by the energy-protein content in diets or by different environmental conditions. When the protein level of the diets is high, animals enhance gluconeogenesis by amino acids from protein degradation; on the contrary, when the protein level of the diets is low, animals reduce production (meat and milk) and afterwards enhance hepatic protein synthesis and the production of microbial protein which may represent a significant part of total amino acid entering the small intestine of host animals. Thus, microbial protein contributes to satisfying the protein requirement of the animal for tissue maintenance and growth and for milk and wool production.

However, as reported for different buffalo species, a NEB is still one of the major concerns that may decrease the productivity in these ruminants and predispose to other pathologies and fertility disorders.

The metabolic profile test in dairy animals is used to assess nutritional status, predict the occurrence of metabolic diseases, diagnose diseases, and assess animal fertility status (Ingraham & Kappus, 1988). Among the factors that influence the metabolic profile in buffaloes, the following were highlighted: nutrition, reproduction, age, sex and climatic factors (Patel et al., 2016; Enculescu et al., 2017). A food deficiency causes metabolic, endocrine and nervous disorders, disrupting hypothalamic-pituitary-ovarian activity, with negative effects on the reproductive process, oogenesis and folliculogenesis, extending the postpartum anestrous period and decreasing fertility indices in cows (Fabry, 1993; Ferguson, 1991; Ghergariu, 1990).

Data on the metabolic profile test in Romanian Buffalo are limited, as there are no available references on normal blood metabolite values (Serdaru et al., 2011). In this metabolic profile research is necessary to continue on a larger number of animals with different sex, age, physiological state and health status (Enculescu et al., 2017).

Immediately before parturition as well as during the first stage of lactation, increased mammary gland activity results in energy deficiency and increased lipomobilization from body reserves. Despite the action of homeostatic mechanisms to maintain blood parameters within physiologic levels, changes in metabolites and hormones occur as a result of increased metabolic demands during both pregnancy and lactation (Ingraham & Kappus, 1988; Fabry, 1993). These changes are not necessarily indicative of diseases but make pregnant animals physiologically unstable and more susceptible to a number of metabolic diseases at this stage than during other life periods compromising productivity (Ferguson, 1991). Homeostasis control involves maintenance of physiological equilibrium or constancy of environmental conditions within the animal. Homeostasis is the orchestrated or coordinated control in metabolism of body tissues necessary to support a physiological state (Ghergariu, 1990). Peripartum period represents a critical life phase in buffaloes as well, since have to adjust metabolically to the increase in energy and nutrient requirements needed to ensure milk production (Ghergariu, 1990). Many authors investigated the buffalo metabolic response to lactation since buffaloes show a different pattern compared to other ruminants as demonstrated by the low incidence of metabolic disorders. The centre of animal physiology is the homeostasis of the glucose, which involves primarily the somatotropine, insulin, glucagon and glucocorticoids with thyroid hormones being usually decreased after calving, with the aim to reduce tissue metabolism in order to have a higher nutrient availability for mammary gland metabolism.

In all species glucose is used by various tissues and organs for free energy (i.e. ATP) production. In addition, glucose may be converted either into glycogen or triacylglycerols which are subsequently stored within tissues (liver, adipose tissues, muscles)

or into lactose which is subsequently incorporated into milk in the case of lactating females. The destination of glucose is regulated by various hormones such as insulin, cortisol, glucagone, somatotropin and adrenalin, and consequently blood glucose levels depend on the nutritive values of the diets, on social or environmental stress conditions as well as on physiological phases.

It has been reported that nutritional deficiency is the important factor responsible for infertility in buffaloes.

As minerals and trace elements such as copper, cobalt, manganese, zinc, etc., play important role in the proper functioning of the genital organs and related activities (Fabry, 1993). Trace elements may function as cofactors, as activators of enzymes or stabilizers of secondary molecular structure (Ghergariu, 1990). Buffaloes are frequently subjected to severe dietary deficiencies of trace elements such as copper, cobalt, selenium, iodine, manganese, and zinc.

In buffalo species calcium excesses could alter the Ca/P ratio during the dry milk period, inducing parathyroid hypoactivity which would cause magnesium to increase and calcium to decrease at the beginning of the lactation due to a non immediate calcium mobilization by the bones. The altered Ca/Mg ratio favours utero-vaginal muscular release, responsible for uterus atony and eventually uterine prolapse Concomitant infertility in buffalo is believed to be associated with enzymatic dysfunctions resulting from these deficiencies (Ghergariu, 1990). Optimum protein level is necessary for the development of endocrine and sex organs.

Deficiency of trace minerals especially calcium, phosphorus and magnesium also influence the ability of animals to utilize other micro-minerals. The influence of these minerals on certain enzyme system may affect reproductive efficiency.

MATERIALS AND METHODS

The aim of this work was to investigate the metabolic profile of buffaloes with disorders of the reproductive cycle and in which estrus was induced through therapeutic protocols, compared to buffaloes that exhibited normal estrus.

The study was carried out at the Research and Development Station for Buffalo Breeding, Sercaia, during 2020, on buffaloes. The Development Research Station for Buffalo Breeding, is located on the national highway DN 1, at kilometer 223, in the territory of the Șercaia municipality, between the towns of Șercaia and Mândra, in the western part of the town of Șercaia, 56 km from the municipality of Brașov. From a mathematical point of view, it falls within 450 11'02"- 450 53'52" northern latitude and 250 06'18"- 250 11'02" eastern longitude. S.C.D.C.B. Șercaia was established in 1981 and it was populated with young female colected from all Romania, also a number of 42 bufflaoes, Murrah breed, was imported from Bulgaria (males and females) at the end of 1983 the herd reached 720 heads.

The herd of buffaloes, from its inception until now, has been the study base for research, the results of which have practical applicability in all buffalo breeding areas in the country, mainly through the diffusion of breeding material in the areas of influence.

Forty female buffaloes, aged between 7 and 13 years, in good maintenance condition and in different physiological states, were divided into two groups (20 buffaloes/group). The first group consisted of buffaloes with disorders of the reproductive cycle that are more than 60 days after calving (active anestrus caused by the presence of the corpus luteum and passive anestrus without the corpus luteum on the ovary) to which specific induction treatments were applied and estrus synchronization. The second group consisted of female buffaloes that showed normal oestrus after calving.

The buffaloes in both groups were maintained both in free housing and in the shelter being fed uniformly with green fodder, hay, corn silage and concentrates. When artificial insemination was performed, blood samples were collected. Blood samples (5 to 10 mL) were taken from the jugular vein in the morning. Blood was transported to the laboratory on ice, where serum was extracted and stored at -20ºC until further analysis. From the serum samples, the following were determined by spectrophotometric and enzymatic colorimetric methods: haemoglobin (g/dL), total serum protein (g/dL), phosphorus (mg/dL), calcium (mg/dL), alkaline phosphatase (UI/L),

magnesium (mg/dL), cholesterol (mg/dL), glucose (mg/dL), lipase (UI/L) and urea (mg/dL), using commercial kits (Span Diagnostics) according to protocol the manufacturer.

Data obtained from biochemical estimations were expressed as mean ± standard error (X ± SD) and coefficients of variation (CV) for each group of buffaloes. The data were statistically analyzed by applying the Student's t-test to obtain the significance of the difference of the mean values of the two groups ($p < 0.05$ was considered statistically significant and 95% confidence).

The experimental procedures were carried out in accordance with the Romanian Legislation no. 43/2014 and Council Directive 2010/63/EU on the protection of animals used for scientific purposes.

RESULTS AND DISCUSSIONS

The results of laboratory determinations in buffaloes from the two groups are presented in Table 1.

Table 1. Results of blood biochemical parameters in buffaloes from the two experimental groups

Blood parameters	Group 1 N = 20		Group 2 N = 20		t-test
	X ± SD	CV%	X ± SD	CV%	
Hemoglobin (g/dL)	9.73± 1.02	14.37	12.68± 0,73	20.12	p < 0.05
Total serum protein (g/dL)	6.12± 0.36	22.16	8.40±0.56	17.46	p < 0.05
Calcium (mg/dL)	10.52± 0.35	24.35	8.28±0.37	18.27	p < 0.05
Phosphorus (mg/dL)	3.64± 0.48	18.42	4.27±0.77	11.31	p < 0.05
Magnesium (mg/dL)	3.06± 1.22	6.83	3.63±1.32	8.46	ns
Alkaline phosphatase (UI/L)	181.95 ± 53.17	23.17	175.19± 48.29	27.36	ns
Cholesterol (mg/dL)	98.52± 12.63	14.72	93.40± 10.84	18.57	ns
Glucose (mg/dL)	42.18± 2.62	34.58	54.18± 4.95	26.12	p < 0.05
Lypase (UI/L)	7.76± 2.45	14.38	7.12±2.90	16.74	ns
Uree (mg/dL)	40.80± 5.77	46.17	38.20± 4.49	35.92	ns

X ± SD = mean ± standard error; CV = coefficient of variation; t-test = Student's t-test; ns = not significant ($p > 0.05$).

The hemoglobin level was 9.73 ± 1.02 g/dL in group 1 and 12.68±0.73 g/dL in group 2. The results showed a significant variation ($p < 0.05$) in the hemoglobin level in buffaloes that showed normal estrus before of buffaloes with prolonged anestrus. The higher hemoglobin concentration in buffaloes that exhibited normal estrus can also be attributed to the fact that an animal requires more oxygen in any stressful condition and consequently the hemoglobin concentration can rise (Fagiolo et al., 2004). Although the level of hemoglobin does not directly influence reproductive disorders, still a low value of it could indirectly affect the functioning of the reproductive organs. A low level of hemoglobin influences the oxygenation of the tissues of the reproductive organs, which in turn could affect the cycling (Sharad et al., 2010).

Total serum protein recorded values of 6.12 ± 0.36 g/dL in group 1 and 8.40 ± 0.56 g/dL in group 2. A significant difference ($p<0.05$) was recorded in buffaloes in group 2. Protein deficiency causes uterine subinvolution which leads to prolonged anestrus after calving. Excess protein increases the incidence of retained fetal adnexa, genital infections and low fecundity. Serum calcium was 10.52 ± 0.35 mg/dL in group 1 and 8.28 ± 0.37 mg/dL in group 2 and was within the limits described in the specialized literature by other authors (8-10.8 mg/dL) (Ghergariu, 1990; Sharad et al., 2010; Enculescu et al., 2017). A significantly greater difference in calcium concentration ($p < 0.05$) was recorded in buffaloes from group 1. Serum phosphorus level in anestrous buffaloes (3.64 ± 0.48 mg/dL) was significantly lower ($p<0.05$) than that of buffaloes with normal cycle (4.27 ± 0.77 mg/dL). The lower calcium level in G2 buffaloes is influenced by the existence of lactation (Patel et al., 2016). Ca:P ratio for buffaloes with normal cycle was lower (1.94:1) compared to buffaloes with anestrus (2.89:1). Ca:P ratio should be 2:1 for better reproduction (Sharad et al., 2010). Serum magnesium (G1 - 3.06 ± 1.22 mg/dL; G2 - 3.63 ± 1.32 mg/dL) was within the physiological limits described in the specialized literature for buffaloes (1.8-3.8 mg/dL) (Ghergariu, 1990; Patel et al., 2016; Sharad et al., 2010; Enculescu et al., 2017). Magnesium plays a vital role in the metabolism of carbohydrates, lipids, nucleic acids and proteins. Alkaline phosphatase (ALP) (G1 - 181.95 ± 53.17 IU/L; G2 - 175.19 ± 48.29 IU/L), had values within the physiological limits in buffaloes (Ghergariu,

1990). The individual cholesterol values in buffaloes from both groups did not have significant differences (G1 - 98.52 ± 12.63 mg/dL; G2 - 93.40 ± 10.84 mg/dL), the reference range in the specialized literature being 73-280 mg/dL (Ghergariu, 1990; Patel et al., 2016; Enculescu et al., 2017). In the present study, buffaloes with normal cycle had significantly higher glucose concentration - $p<0.05$, (54.18 ± 4.95 mg/dL) compared to buffaloes with anestrus (42.18 ± 2.62 mg/dL). Several authors have supported the opinion that glucose concentration reflects the energy status and reproductive activity of animals (Sharad et al., 2010; Popa, 2018). Hypoglycemia influences ovarian activity in animals by reducing the release of gonadotrophins from the hypothalamus (Sharad et al., 2010). Blood glucose variations in buffaloes are related to cycling and fertility (Sharad et al., 2010). Serum lipase level was 7.76 ± 2.45 IU/L in G1 buffaloes and 7.12 ± 2.90 IU/L in G2 buffaloes. Reference values of serum lipase are not presented in the specialized literature for buffaloes. Lipase is a key enzyme in triglyceride metabolism. Urea recorded values in the physiological thresholds (21-53 mg/dL) in buffaloes from both experimental groups.

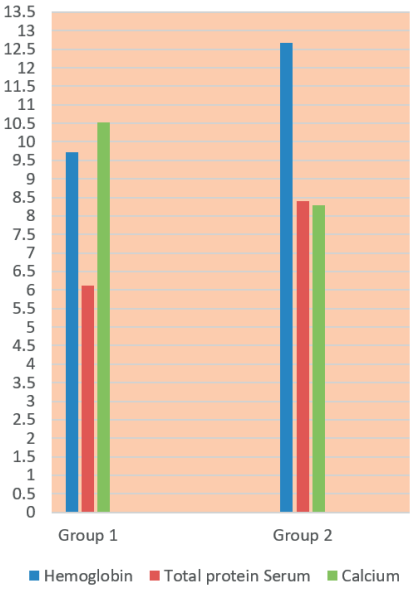

Figure 1. Results regarding hemoglobin, total protein serum and calcium

CONCLUSIONS

The metabolic profile is a beneficial means that has progressed over time. This progression or adaptation is very important to be consider for changes in feeding management.

Serum biochemical and haematological reference values are used to establish normality and to diagnose disease and physiological alterations. It could be concluded that reference values for haematological and serum biochemical constituents in lactating buffaloes were estimated in this study, these values will be helpful in interpreting laboratory results, diagnosis of diseases, and checking the health status.

From the present study it can be concluded that the levels of haemoglobin, serum proteins, glucose, calcium, inorganic phosphorus play an important role in the reproduction of buffaloes. The Ca:P ratio should also be close to 2:1 for better reproductive system activity. Subclinical nutrient deficiencies are a cause of clinical anestry in buffaloes.

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