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Original article

Relationship of body condition score and blood urea and ammonia to pregnancy in Italian Mediterranean buffaloes

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Abstract – The relationship of body condition score (BCS) and blood urea and ammonia to pregnancy outcome was examined in Italian Mediterranean Buffalo cows mated by AI. The study was conducted on 150 buffaloes at 145 ± 83 days in milk that were fed a diet comprising 14.8% crude protein, 0.9 milk forage units·kg^{-1} dry matter and a non-structural carbohydrate/crude protein ratio of 2.14. The stage of the oestrous cycle was synchronised by the Ovsynch-TAI programme and blood urea and ammonia levels were assessed on the day of AI. Energy corrected milk (ECM) production and BCS were recorded bi-weekly. The pregnancy risk was 46.7% and was slightly lower in buffaloes with BCS < 6.0 and BCS > 7.5. There were no significant differences in ECM, urea and ammonia between pregnant and non-pregnant buffaloes. However, pregnancy outcome was higher (P = 0.02) in buffaloes with blood urea < 6.83 mmol·L^{-1}. The likelihood of pregnancy for buffaloes with low urea blood level was 2.6 greater than for high urea level and exposure to a high urea level lowered the probability of pregnancy by about 0.25. The findings indicate that buffaloes are similar to cattle and increased blood levels of urea are associated with reduced fertility when animals are mated by AI.

buffalo / BCS / urea / ammonia / pregnancy

1. INTRODUCTION

Reproductive function in buffaloes during the post-partum period is influenced by a number of factors that include body condition and dietary status. Buffaloes with an optimal body condition at calving have a reduced calving to conception interval due to an earlier resumption of cyclic ovarian activity and fewer services per conception [1, 2]. The importance of an optimal body condition has been demonstrated by reduced fertility in buffaloes that were overtly fat at the time of calving [2]. Energy balance is also important and buffaloes in negative energy balance showed a reduced ovarian

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follicular activity and a delay to ovulation post-partum [3]. The quantity of protein is an additional factor that can influence ovarian activity and uterine function in buffaloes. Italian Mediterranean Buffaloes fed a diet with protein content 50% greater than requirements had higher incidences of oestrus that were followed by a normal luteal phase and typical inter-oestrous interval [4]. In the contrast to the quantity of protein, degradability in the rumen has no apparent effects on reproductive performance in buffaloes mated naturally [5]. It has been suggested that the failure of rumen protein degradability to influence fertility in buffaloes is due to a lower uterine diffusion of ammonia in buffaloes compared with cattle [5]. The aim in the present study was to obtain clear relationships between body condition, blood levels of urea and ammonia, and risk for pregnancy in Italian Mediterranean Buffaloes synchronised and mated by AI in the mid-winter. The latter coincided with transition to the seasonal nadir in reproductive function in buffaloes.

2. MATERIALS AND METHODS

2.1. Animals and diet

The study was conducted using multiparous, Italian Mediterranean Buffalo cows (n = 150) at 145 ± 83 (mean ± SD) days of lactation. The buffaloes were maintained in communal open yards that allowed 15 m^2 for each animal with free choice access to feed. The ingredients and chemical composition of the diet, administered once a day in the morning as a total mixed ration (TMR), are shown in Table I. Feed intake was determined from orts (refusals) collected daily in the morning before the next feed administration. The amount and the composition of orts were utilised to calculate the dry matter (DM) intake and the composition of the ingested diet. Individual feedstuff, TMR and orts were carried out as per AOAC (Association of Official Analytical Chemists) methods [6] and energy values (Milk Forage Units – MFU = 1700 kcal NEL) were calculated according to INRA (Institut National de la Recherche Agronomique) equations [7]. The animals were weighed at the beginning and end of the study. Body condition score (BCS) was recorded on the day of AI on a scale of 1 to 9 [8] and the animals were grouped in the following categories: lean (< 6.0); optimal (6.0–7.5); fat (> 7.5).

Table I. Composition of the daily diet.

Dry matter	15.2 ± 0.9	(kg)
CP	14.8 ± 0.6	(% DM)
NDF	44.3 ± 3.9	(% DM)
ADF	22.4 ± 2.1	(% DM)
NSC	31.6 ± 2.3	(% DM)
Starch	19.9 ± 2.5	(% DM)
MFU	0.90 ± 0.1	(% DM)
NSC/CP	2.1 ± 0.2	
RUP/CP	31.6 ± 5.1	(%)
RDP/CP	68.4 ± 4.2	(%)
SIP/CP	32.8 ± 4.6	(%)

CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber), NSC (non-structural carbohydrates), MFU (milk forage unit), RUP (rumen indigestible proteins), RDP (rumen digestible proteins), SOL (soluble proteins).

2.2. Milk sampling, analytical methods, standard milk, and calculation of differences between intake and requirements

Milk yield was recorded fortnightly for 60 days and, at the same time, a sample was taken at the morning and afternoon milkings to determine milk fat and protein percentage using infrared spectroscopy (Milkoscan 139, Foss Electric, Hillerød, DK) calibrated with the appropriate buffalo standard. Buffalo standard milk (energy corrected milk (ECM) = 740 kcal) was calculated using the formula for buffalo cows: $([\{\text{fat}\,(\text{g}\cdot\text{kg}^{-1}) - 40 + \text{protein}\,(\text{g}\cdot\text{kg}^{-1}) - 31\} \times 0.01155] + 1) \times$ milk yield.

2.3. Oestrus synchronisation, AI and pregnancy diagnosis

The buffaloes were subjected to a clinical examination of the reproductive tract and ovaries and only animals with a corpus luteum and/or follicle ≥ 1 cm were selected for synchronisation of the stage of the oestrous cycle and AI. The synchronisation protocol used (Ovsynch-TAI Program) was similar to that developed for cattle [9] and previously applied in buffaloes [10]. Briefly, it consists of Day 0, GnRH agonist (buserelin acetate, 12 μg; Receptal®, Intervet); Day 7, PGF_2 analogue (luprostiol, 15 mg; Prosolvin®, Intervet); Day 9, GnRH agonist (12 μg). Artificial insemination was performed by the same operator and each buffalo was inseminated twice, 16 h and 40 h after the second injection of the GnRH agonist (period Jan–Mar 2002). Because of the relatively low intensity of oestrous behaviour in buffaloes [11], the animals were palpated per rectum (immediately before AI) to assess oestrous status (follicle 1.0 cm and a tonic uterus with the presence or absence of a mucous vaginal discharge). Pregnancy diagnosis was carried out on Day 40 after AI using trans-rectal ultrasonography. The latter was conducted with an Aloka SSD-500 equipped with a 5.0 MHz linear array probe (Aloka CO., Tokyo, Japan) and was carried out by the same experienced operator.

2.4. Blood samples and ammonia and urea analyses

On the day of AI, a blood sample was taken from the jugular vein of each buffalo before feeding. Samples were centrifuged at 3000 *g* for 15 min and serum was stored at –18 °C until required for analyses. Immediately after sampling, the ammonia (NH_3) was measured on whole blood with a rapid method (Ammonia kit, Menarini, Florence, Italy) that has a high correlation ($r = 0.988$) with the enzymatic UV-method [5]. The intra-assay coefficient of variation of the ammonia rapid method was 7.7%. Urea was measured [12] in serum using the enzymatic colorimetric method (urease method – SCM, Rome, Italy).

2.5. Statistical analyses

A multivariate logistic regression analysis [13] was performed with the dependent variable of interest defined as the risk for pregnancy (pregnancy outcome: 0, non-pregnant; 1, pregnant). The independent (explanatory) variables used in the model were blood ammonia and urea levels and BCS. The first two variables were introduced in the model as linear and non-linear terms (e.g. quadratic and cubic) and also categorised according to the range considered physiological for the species. Levels were set for blood urea (0, < 6.83 $mmol \cdot L^{-1}$; 1, ≥ 6.83 $mmol \cdot L^{-1}$) and blood ammonia (0, < 46.98 $\mu mol \cdot L^{-1}$; 1, ≥ 46.98 $\mu mol \cdot L^{-1}$). Categorisation in concentration ranges was used to verify if the levels above those stated as physiological [14] were deleterious. It is likely that pregnancy is linked to the exposure to urea or ammonia above the threshold levels with an all or none effect. The number of days in milk (DIM) at AI, level of milk production (expressed both as milk and ECM yields), the three month period of AI (categorical) and parity were used as covariates in order to verify their effects as potential confounders. The odds ratios for the variable in the model and the attributable risk of pregnancy were calculated.

Multiple regression analysis [13] was used to evaluate the relationship between blood urea and ammonia with BCS as an explanatory variable. Data for DIM and milk and ECM yields were used in the model in order to avoid confounding effects. BCS was inserted in the model as a dummy variable with three levels (Level 1 (lean), < 6.0; Level 2 (optimal), 6.0–7.5; Level 3 (fat), > 7.5).

3. RESULTS

The daily DM intake during the course of the study was 15.2 ± 0.9 kg (Tab. I). The CP/DM was 0.3% lower while the MFU/DM and non-structural carbohydrates

Table II. Blood levels of ammonia and urea, equivalent corrected milk (ECM) production, and pregnancy risk, in buffaloes characterised by BCS (Level 1 (lean), < 6.0; Level 2 (optimal), 6.0–7.5; Level 3 (fat), > 7.5). The results are means ± SD.

BCS	*n*	Ammonia ($\mu g \cdot dL^{-1}$)	Urea ($mmol \cdot L^{-1}$)	ECM (kg)	Pregnancy risk (%)
Level 1	9	80.9 ± 11.3	8.6 ± 0.6	14.4 ± 1.7	33.3
Level 2	122	44.7 ± 3.0	7.8 ± 0.1	14.2 ± 0.4	49.2
Level 3	19	62.4 ± 7.4	7.4 ± 0.2	14.6 ± 1.1	36.8

Table III. Days in milk (DIM), body condition score (BCS), milk yield, percentage of milk fat and protein, ECM and blood levels of urea and ammonia in pregnant (P) and non-pregnant (NP) buffaloes. The results are means ± SD. There were no significant differences for any of the parameters between P and NP buffaloes.

Parameter	P		NP	
	n		*n*	
DIM	70	141 ± 76	80	148 ± 89
BCS	70	6.8 ± 0.9	80	6.9 ± 0.8
Milk yield (kg)	70	8.4 ± 3.8	80	8.3 ± 3.4
Fat (%)	68	8.5 ± 1.6	80	8.5 ± 1.5
Protein (%)	68	4.8 ± 0.4	80	4.9 ± 0.4
ECM (kg)	68	14.7 ± 5.8	80	14.3 ± 5.9
Blood urea ($mmol \cdot L^{-1}$)	64	7.7 ± 1.1	74	7.8 ± 1.0
Blood ammonia ($\mu mol \cdot L^{-1}$)	68	28.2 ± 19.4	79	30.8 ± 21.7

(NSC/DM) were, respectively, 0.3% and 2.0% higher than the requirements recommended for buffalo milk production [15]. Similarly, buffaloes received 20% more energy (ΔMFU = + 2.2) and 46% more protein (ΔCP = + 706 g) than recommended.

Ammonia blood levels showed a significant ($P < 0.01$) relationship with BCS (Level 1, to Level 3) and ECM (Tab. II) as shown in the following equation:

$$NH_3\ (\mu mmol \cdot L^{-1}) = 30.56 - 14.47\ \text{Level 2 (BCS)} + 0.56\ \text{ECM}\ (R = 0.417).$$

No relationship was found between blood urea levels and BCS.

All buffaloes had a follicle 1.0 cm and received AI. The pregnancy risk on Day 40 after AI was 46.7% (70/150).

Data for DIM, BCS, urea, ammonia and milk yield and ECM yield for pregnant and non-pregnant buffaloes are shown in Table III. The multivariate logistic regression analyses showed no significant effect of confounding variables such as milk and ECM yields, parity and DIM on pregnancy outcome. Similarly, no effect of BCS was found on pregnancy outcome. The lowest pregnancy outcome did, however, occur in lean buffaloes (BCS Level 1 < 6.0) on the day of AI, although this was not significant (Tab. II). There was no significant effect of blood levels of ammonia on pregnancy outcome while a negative effect ($P = 0.02$) of high urea blood level was found (Tab. II). With regards to the latter, the negative effect existed only for categorised urea levels and the odds ratio for pregnancy outcome linked to high urea level was 2.60 (95%

confidence interval: 1.14–5.97). The attributable outcome for pregnancy risk linked to the exposure to high blood urea level was 0.25 (which quantifies how the proportion of not pregnant buffaloes increases, due to high urea blood levels).

4. DISCUSSION

Synchronisation of the stage of the oestrous cycle with the Ovsynch-TAI Programme and fixed-time AI resulted in an overall pregnancy outcome of 46.7% which was relatively high for buffaloes mated in the mid-winter [10–16]. In contrast to previous reports in buffaloes [2] and cattle [17] there was no apparent relationship between BCS and pregnancy outcome in the present study. The present study was conducted at a time of increasing day length which tends to suppress fertility in female buffaloes and this may have masked or overridden any potential relationships with nutrition. However, the majority of buffaloes (80%) in the present study were in optimal body condition.

In a study that involved natural mating of buffaloes during a period of increasing day length, blood levels of urea > 6.66 mmol·L^{-1} did not influence fertility [5]. In the present study, blood levels of urea ≥ 6.83 mmol·L^{-1} were associated with a significantly reduced likelihood of pregnancy. The likelihood of pregnancy for buffaloes with low urea blood level was 2.6 greater than for a high urea level and exposure to a high urea level lowered the probability of pregnancy by about 0.25. A difference between the previous [5] and present studies was the use of frozen semen in the latter. Urea appears to inhibit the citric acid cycle in sperm and compromises motility and fertilising ability, effects that are likely to be more pronounced in cryopreserved sperm [18]. It is also possible that urea may have influenced fertility in the present study by negative actions at the ovaries. In this regard, serum urea levels > 5.83 mmol·L^{-1} caused a reduction in progesterone secretion in dairy cows and this was associated with a decline in pregnancy [19]. The blood levels of urea observed in the present study were relatively high compared with previous reports in buffaloes and cattle [5, 20] which could be explained by the surplus dietary protein provided in the present study.

It has previously been shown that excess dietary protein in buffaloes increases the blood levels of urea but not ammonia [5]. This is in marked contrast to cattle in which a relatively modest increase in protein causes ammonia to increase in the rumen, with associated increases in ammonia and urea in the blood and milk [21]. Buffaloes are able to utilise nitrogen more efficiently than cattle since the rumen environment in buffaloes is favourable to non-protein nitrogen utilising micro-organisms [22]. Also, the liver in buffaloes effectively converts ammonia, a toxic metabolite, into urea which can be either utilised by rumen micro-organisms or eliminated without detrimental effects [5]. An increase in dietary protein intake raises the metabolic activity of hepatic microsomes which favours the transformation of alimentary ammonia into urea [23].

Blood levels of ammonia observed in the present study were lower than those reported in a previous study in which buffaloes received a diet with 26% protein surplus [5]. It is possible that in the present study a better utilisation of ammonia in the rumen occurred due to a more favourable ratio between NSC and CP (2.14) compared with the ratio (1.86) in our previous study [5]. Under the conditions of the present study, microbial activity in the rumen would be increased. In fact, improved rumen activity due to high NDF values and a lower digestible protein intake reduces the production of ammonia in the rumen and hence in the blood.

5. CONCLUSIONS

Buffaloes are similar to cattle in that increased blood levels of urea are associated with reduced fertility. The lack of an apparent relationship between BCS at the time of AI and pregnancy could be interpreted to suggest that the effects of nutrition and

metabolic status on fertility in female buffaloes can be influenced by photoperiod.

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