Nutrient Requirements of Exercising Swamp Buffalo, *Bubalus bubalis*, from Materials Balance and *In Vivo* Body Composition by the Body Density Method. I. Aspects of Energy and Protein Metabolism in Working Cows[a]

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ABSTRACT : Four young swamp buffalo cows of similar age ranging in weight between 280 to 380 kg and trained to do physical work were used in a study to determine energy and protein requirements for draught using a 4×4 Latin square designed experiment. The experiment consisted of field trials employing 4 levels of work load, e.g. no work as control, and loads amounting 450 to 500 Newton (N) pulled continuously for 1, 2 and 3 h daily for 14 consecutive days. Cows were fed king grass (*Penisetum purpuroides*) *ad libitum* and were subjected to materials balance trials. Body composition was estimated *in vivo* by the body density method and daily energy expenditure (EE) was calculated from ME minus retained energy (RE). The results show that EE while not working ($EE_{resting}$) was 0.42 $kgW^{0.75}$ MJ/d and maintenance ME (ME_m) was 0.37 $kgW^{0.75}$ MJ/d. ME requirement increased to 1.65 times maintenance for the work of 3 hours. The energy expended for doing exercise ($E_{exercise}$) was 9.56, 20.0 and 25.86 MJ/cow for treatments 1, 2 and 3 h, respectively. Fat retention was absent in all groups of working cows, but protein retention was only negative for cows undertaking 3 h work. The relationship between $E_{exercise}$ (MJ), work load (F, kN), work duration (t, h) and body mass (W, kg) was found to be: $E_{exercise}=(0.003F^{1.43}t^{0.93})/W^{0.09}$ MJ. The maintenance requirement for digestible protein was 2.51 $kgW^{0.75}$ g/d, whereas digestible protein for growth (DP_{growth}) and for work (DP_{work}) followed the equations: $DP_{growth}=[(258+1.25W^{0.75})\Delta Wkg/d]g$ and $DP_{work}=[12.59e^{0.95t}]g$, respectively The coefficients a, b and c for the calculation of $E_{exercise}$ components according to the Lawrence equation were found to be 2.56 J/kgW.m, 5.2 J/kg load carried.m and 0.29, respectively, thus efficiencies to convert ME into work were 0, 16.09, 27.3 and 32.44% for control, 1, 2 and 3 h/d work, respectively. ME and DP requirements for a 250 to 400 kg working buffalo cow allowing to growth up to 0.5 kg/d are presented. (***Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 5 : 605-612***)

Key Words : Swamp Buffalo, Field Balance Trial, ME and Protein Requirements, Exercise, *In Vivo* Body Composition

INTRODUCTION

Draught animals are still important for providing the power needed in developing countries where feed resources are usually inadequate. Adequate feeding is necessary, and information is needed on nutrient utilization, especially for the humid tropical condition. It is customary believed that nutrient requirement of draught animals in terms of energy for maintenance is the same as the maintenance demand for other productive purposes. Hence, rationing becomes simple by supplementing the extra energy needed for work on top of the feed energy required to maintain the animal at a stable live weight. From information on the energy needed for work and the heat increment associated with work, the extra metabolizable energy (ME) for work can be calculated and be translated into quantities of food (Lawrence, 1985). The most suitable method for measuring energy for work under field conditions is the factorial method developed by Lawrence and associates at CTVM, University of Edinburgh, UK, requiring measurements of live weight of the animal, its walking speed and the draught force exerted (Lawrence, 1985; Lawrence and Stibbards, 1990; Bakrie and Masum, 1993). Lawrence (1985) pointed out the difficulty in doing field trials to measure work and suggested the need to measure energy expenditure (EE) of animals for 24 hours both while at rest and when doing a normal day's work or other form of physical exercise. These measurements would involve the use of an animal chamber or the face mask technique. The methods entail restrictions on the freedom of the experimental subject; the chamber method precludes free living estimation while the face mask method is limited to short term determinations requiring some subject co-operation and

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appropriate expired gas sampling protocols to enable the calculation for total 24 h values from individual recordings (Clar et al., 1992). In addition, it is unsuitable for application on free fast moving animals. An alternative non-invasive method is therefore imperative.

The purpose of the present study was to measure the energy and protein balance of working swamp buffalo cows in the field in order to determine energy and protein requirements. The experimental approach was to estimate daily EE, which includes that for physical exercise, of exercising buffalo from an energy balance within a 14 day trial using the relationship that EE is equal to ME minus retained energy (RE). RE was calculated from daily gain of body weight and body composition estimated by the *in vivo* "body density/water displacement" method (Kleiber, 1961). Preliminary results of the study were presented at the 14th symposium on energy metabolism of farm animals in Newcastle, Co. Down, Northern Ireland (Mahardika et al., 1998).

MATERIALS AND METHODS

Experimental design and protocol

Four non-pregnant, non-lactating swamp buffalo cows weighing 280 to 350 kg which had been trained to do daily exercise were used for the study. The training program was to pull loads on a sledge continuously for 3 h during a period of 2 to 3 months. The health of animals was monitored, based on physical examination and hematological data prior to the experiment. Ranges of blood values were 13.4 to 18.8 g/dl for Hb, 5.5 to $6.7\times10^6\,\mu l^{-1}$ RBC, 34 to 47% PCV, 8.5 to $12.2\times10^3\,\mu l^{-1}$ WBC and pH 7.36 to 7.43. When the buffaloes were not doing field exercise, they were placed in individual pens with facilities required for balance trials. Measured amounts of king grass (*Penisetum purpuroides*) aged 45 to 55 days as the sole feed were given *ad libitum*. King grass contained 89.3% DM, and on a DM basis, 11.7% CP, 4.9% fat, 20.8% CF, 44.5% NFE, 7.5% ash, and 65.9% TDN (Sumaryadi and Manalu, 1999). Water in excess was offered twice a day at noon and in the evening.

The design of the experiment was a 4×4 latin square with periods lasting for 3 weeks. The field trials involved pulling loads of 450 to 500 Newton (N) daily on a flat circular track (114 m circumference) for 1, 2 and 3 h continuously for 14 consecutive days starting at 7.00 a.m (4 treatments including non-working as control), beginning on day 8 of each experimental period. A sledge which was the traction equipment loaded with the driver and weights was furnished with a traction scale to measure draught force; a distance measuring device and a stop watch to quantitated the treatment load. When pulling, the animals wore a single shoulder yoke; the angle between traction force and horizontal level (α) was carefully recorded. Readings of treatment loads were recorded by visual observation at 2 minute intervals during 30 min at the start (from minute 0 to 30 of the traction period), during 30 min in the middle (from minute 75 to 105) and during 30 min before termination of the exercise (from minute 150 to 180). The sledge is shown in figure 1. The ground covered with a thin layer of sand was hard and dry. The track was located in a shady field under coconut trees with air temperatures ranging from 24 to 32℃ and 70 to 95% relative humidity during the morning hours of the trials. Solar radiation was between 60 and 70%.

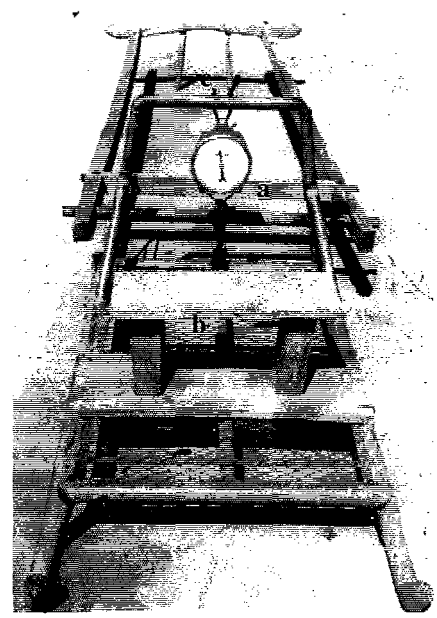

Figure 1. The traction equipment consists of a sledge with a force measuring device (a), a chair (b) for the driver and loads, and a yoke (c). The yoke is connected to the sledge by a rope attached via a pulley to the force measuring device.

Using a 1000 kg maximum capacity weighing scale, the cows were weighed weekly in the morning for 3 consecutive days and the average value was taken. Excreta and refused feed were collected before morning feeding, recorded weights and sampled for proximate analysis. Energy contents of feed and excreta were measured by bomb calorimetry using a Gallenkamp calorimeter. ME was calculated as the difference between gross energy (GE) intake and total energies in feces, urine and methane. Energy loss as methane ($E_{methane}$) was estimated according to Blaxter (1969) using the equation: $E_{methane}$ (kJ/100kJ feed)= 4.28+0.059D (energy digestibility in %).

Body composition and measurement of daily EE

Body composition was determined *in vivo* by the mass densitometric method in water according to Kleiber (1961) using the relationship $W_f/W_b=[v_b-v_l]/[v_f-v_l]$, where W_b=body mass (kg), W_f=fat mass (kg), W_l=lean mass (kg), v_b, v_l and v_f were specific volumes (ml/g) of body, lean and fat mass, respectively. To measure the animal's volume, the animal was carefully lowered and immersed in a concrete basin fully filled with water measuring 2 m (length)×1.0 m (width)×1.0 m (depth) constructed in the ground with a sloping entrance. The overflow of water was totally collected in a special constructed container beside the basin and measured to estimate the animal's body volume. Our standard procedure was to immerse the whole buffalo body leaving only the nostrils above water level, which took about 2 to 3 minutes without resistance from the animal who loves wading in water, thus allowing displaced water, as far as we could tell, to overflow completely.

Body protein was calculated using an equation relating to the ratio of meat to bone (R_d), which are the components of lean minus blood, and body weight (W in kg), according to the equation: R_d=2.861+0.0109W (r^2=0.99; SD=0.27) derived from swamp buffalo data reported by Natasasmita (1978). Body protein was then calculated by multiplying protein percentage of swamp buffalo meat to quantity of meat. For the calculation of body composition of swamp buffalo, the constant v_f=1.110 (for 36℃ body temperature) was used, which was the average value from many buffalo fat samples obtained from the local slaughter house. The value of v_f=1.110 was also reported by Fidanza (1953; cited by Kleiber, 1961) for man and other mammals. Constant v_b was obtained from the density measurement. Body water was measured by urea space (Rule et al., 1986). (Thus, e.g. if measured v_b=0.9497, then v_l=0.915, and if in addition Wb is known, e.g. 302 kg, then W_f=53.466 kg. W_l=248.534 kg and water free lean 69.589 kg, total meat 59.860 kg and body protein 40.705 kg). From the changes in body-protein and -fat measured before and after the 14 d experimental period, RE was calculated assuming energy equivalents of 39.32 MJ/kg and 20.07 MJ/kg for body-fat and -protein, respectively (Mahardika et al., 1998).

The calculated daily heat production while resting ($EE_{resting}$), was obtained from the control data. Energy for exercise ($E_{exercise}$) associated with a particular work load was calculated from EE of the working group ($EE_{working}$) concerned substracted by $EE_{resting}$.

Energy for work exercise in the field

The amount of energy used by the working animal in the field was calculated according to the factorial method (Lawrence, 1985) expressed as :

$$E_{exercise}=aWD+bLD+E_{pull}/c+9.81HW/d$$

where

$E_{exercise}$ = energy used for the work exercise (kJ)
W = liveweight (kg)
D = distance travelled (km)
L = load carried (kg)=Fsinα; F=measured draught force
E_{pull} = work done in pulling a load (kJ)=Fcosα (N)×D (km)
H = distance moved vertically upwards (km)

The original factors a, b and c have been derived from controlled laboratory studies at CTVM being for buffaloes: a=2.09, b=4.24, c=0.38 and d=0.35. These factors have been modified with our own findings (see results and discussion) for the calculations of work energy categories of $E_{exercise}$ in this study.

The work treatments were to pull loads on a flat surface and therefore H=0 and the energies for moving uphill (9.81 HW/d in the equation) were zero. The efficiency of conversion of daily ME into work was calculated as $[E_{exercise}/ME]\times 100\%$.

Statistical analysis

The data obtained were subjected to analysis of variance and if significant differences were obtained between treatments, the data were further analysed by the Duncan Multiple Range Test (Steel and Torrie, 1986). Calculation of mathematical models to relate different measured variables employed multiple regression analysis using Lotus 123 release 5 program.

RESULTS AND DISCUSSION

Table 1 shows mean nutrient intakes and balances, and weight gains of working cows fed king grass. Significant differences ($p<0.05$) among treatments were observed on intakes of DM, GE, DE, ME and protein. DM intakes ranged from 2.02% W (no work) to 2.71% W (3 h work load), but DM digestibilities were not different among treatments. Considering *ad libitum* feeding, the level of energy output i.e. physical activity, is a factor regulating the level of energy intake. Even though work load increases nutrient intakes, live weight gain was reduced with increasing work load. Stall feeding with king grass only imposed nutritional limitation and requirements were not met with the increasing demand of physical work. From the data on DM intake (DMI), a multiple regression relationship with body mass (W, kg), draught force (F, N) and duration of exercise (t, h) were found:

$DMI=3.86-0.025W^{0.75}+0.0003F+0.39t-0.038t^2$ (r^2=0.83; SD=0.38).

The quadratic relationship between DMI and work duration could explain the reduction in feed

Table 1. Category of energy and protein intakes and retained body constituents in working 280 to 380 kg non-pregnant, non-lactating swamp buffalo cows

Parameter	Daily continuous work load			
	no work	1 hour	2 hours	3 hours
Energy and protein intakes:	*Mean±SE (of 4 replicates)			
DM[1] (% W)	2.02 ±0.088[a]	2.48 ±0.126[b]	2.60 ±0.128[bc]	2.71 ±0.059[c]
GE[2] (MJ/d)	91.77 ±7.086[a]	109.51 ±1.101[b]	134.62 ±5.371[c]	145.06 ±1.246[d]
DM digestibility (%)	50.05 ±2.871[a]	50.27 ±1.616[a]	51.87 ±1.212[a]	51.46 ±0.944[a]
ME[3]/DE[4]	0.77[a]	0.75[a]	0.78[a]	0.77[a]
ME (MJ/d)	35.35 ±2.168[a]	39.73 ±1.641[b]	50.78 ±0.415[c]	55.44 ±1.374[d]
Protein intake (kg/d)	0.56 ±0.029[a]	0.69 ±0.006[b]	0.75 ±0.035[b]	0.71 ±0.006[b]
ADG[5] and retained energy:				
⊿W[6] (kg/d)	0.50 ±0.144[a]	0.18 ±0.054[b]	-0.11 ±0.128[c]	-0.32 ±0.156[d]
Rfat[7] (kg/d)	0.07 ±0.038[a]	-0.04 ±0.009[b]	-0.08 ±0.033[bc]	-0.09 ±0.026[c]
Rprotein[8] (kg/d)	0.106±0.062[a]	0.076±0.012[a]	0.014±0.046[b]	-0.078±0.057[c]
RE[9] (MJ/d)	4.96 ±0.580[a]	0.015±0.362[b]	-2.75 ±1.055[c]	-4.91 ±1.662[d]
Energy expenditure:				
EE[10]=ME-RE (MJ/d)	30.383±2.351[a]	39.715±1.116[b]	53.536±1.101[c]	60.352±1.234[d]

* Means in a row with differing superscripts differ significantly (p<0.05).
[1] DM = daily dry matter intake of feed.
[2] GE = gross energy, daily intake.
[3] ME = metabolizable energy, daily intake.
[4] DE = digestible energy.
[5] ADG = average daily gain of body mass, W.
[6] ⊿W = ADG.
[7] Rfat = daily retained fat.
[8] Rprotein = daily retained protein.
[9] RE = daily retained energy.
[10] EE = daily energy expenditure.

consumption after a certain level of work load. Increase of work load causes increases in heat production and blood glucose concentration, and these in turn depress feed intake as elucidated according to thermostatic and glucostatic theories. Kearl (1982) reported that a 300 kg swamp buffalo gaining weight at 0.25 to 0.50 kg/d would consume feed amounting 2.1 to 2.3% W. The data of the present study show that the non-working cow consumed 2.02% W, gained 0.5 kg/d of body mass, and the 1 h working group consumed 2.48% W and gained 0.18 kg/d. These values are in agreement with Kearl's findings. The working animals receiving 2 and 3 h work loads, on the other hand, lost weight, -0.11 and -0.32 kg/d respectively. A regression equation could be calculated from gain in body mass, ⊿W (kg/d), and duration of daily exercise t (h) as follows: $\Delta W=1.567e^{-0.331t}-1$ (r^2=0.75; SD=0.75).

The negative gains in response to work load suggest that feeding king grass only to the working buffalo is inadequate to meet energy requirements, therefore supplementary feeding should be offered. Changes in ME intake affected by work went parallel to changes in GE intake (table 1), the ratio ranged from 36.33 to 38.56% which is not significantly different among treatments. This was due to DM digestibility, which was between 50.5 and 51.87%, not being affected by work load (Ndlovu, 1995), and ME/DE was also constant, 0.75 to 0.78. Thus, ME (MJ/d) relates to duration of work load t (h):

$$ME=[ME_{max}t^{1.89}]/[0.53+t^{1.89}] \quad (r^2= 0.77, SD=0.46)$$

where calculated ME_{max} equals 59.5 MJ/d.

Data on protein, fat and energy retentions were derived from the *in vivo* body density method in which it is required to measure body volume. The major source of error in measuring body volume by this method is the uncertainty of the estimate of air volume in the lungs and in the gastro-intestinal tract. However, since our interest is to get data on retained fat and protein and not on the absolute composition of the body as such, by measuring the body volume twice at the beginning and at the end of the 14 d balance trial to get the difference, the errors inherent with the individual measurements would be cancelled out for the calculation of the quantitative changes in body components. Our data show that swamp buffalo body fat varied from 16.8 to 18.7% of W and protein 17.4 to 18.7% of W. Up to 3 h/d work did not significantly change body composition. Non-working cows retained 0.07 kg/d fat, while those working lost fat. A similar situation applied also for protein retention, but a negative retention was only observed in cows working 3 h/d. (figure 2). The reduction in fat and protein contents were due to their fate as energy resources for work, with fat degradation occurring earlier than protein degradation (Carlson and Hsieh, 1970). However, prior to protein use, elevated carbohydrate and fat degradations require the involvement of enzymes and hormones as catabolic regulators. Most of these substances are proteins and polypeptides, hence their turnover including degradation should be faster during physical work. In addition, individuals undertaking a prolonged period of physical exercise would require faster repair of their contractile proteins such as muscle proteins, and consequently, a slight increase in protein catabolism would be expected, although not for ATP generation in the first place.

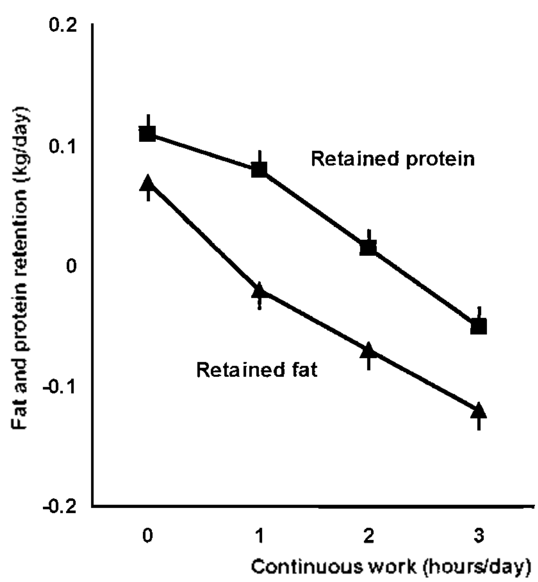

Figure 2. Reduction in body fat and protein retentions of working swamp buffalo cows due to length of daily work (Mean±SE, 4 replicates)

The elevated fat and protein degradation due to work were also reflected in elevated excretion of urinary water due to increased metabolic water production and increased urinary nitrogen, increments being 8.32 liter/d (control) to 19.41 liter/d (3 h work) for urine volume and 15.95 g/d (control) to 35.87 g/d (3 h work) for urinary N. Although not measured in the present experiments, elevated excretion of urinary water may also be caused by increased drinking associated with homeostatic mechanisms of the body, e.g. for thermoregulatory purposes and keeping normal water balance as a result of physical exercise. The relations with time (h) for fat- and protein-retentions [Rfat (kg/d) and Rprotein (kg/d)] in the present study were:

$Rfat=1.05e^{-0.065t}-1$ (r^2=0.70; SD= 0.23)

$Rprotein=1.19\ (t+1)^{-0.18}-1$ (r^2=0.62; SD=0.18)

Skeletal, muscular and organ development during growth are intricately associated with endocrine factors and proper nutrition. Epinephrin has a calorigenic effect in mammals and is closely linked to activity. Epinephrin and nor-epinephrin stimulate the liberation of FFA from adipose tissue. Cortisol has an effect on gluconeogenesis from protein. The utilization of protein in our present study indicated that together with glycerol from triglyceride breakdown, gluconeogenesis was permitted to proceed by provision of substances in the Krebs cycle, thus supporting muscular activity and securing the central nervous system to function optimally during exercise, hence decreasing the chance of fatigue and the risk of serious injury (Powers and Howley, 1991). The data on protein degradation in the present study also support the recent opinion that the total contribution of protein to the fuel supply during exercise may reach values far above 2% depending on exercise duration (Berg and Keul, 1980; Lemon and Nagle, 1980; White and Brooks, 1981). However the present finding is contradictory to the notion that there seems to be very little requirement for extra protein during work (Lawrence, 1985). According to that notion it is believed that if a working animal is not to lose weight, it must consume more energy rich feeds and this will almost certainly involve taking in extra protein as well. Our results clearly demonstrate the contribution of protein in the energy supply of the working animal, thus affirming the requirement for protein in the diet of the swamp buffalo, especially when weight gains are also aimed at. Exercise, even at low intensity, elicited an accelerated release of glucose by the liver that would require a significant increase in ME intake to supply more gluconeogenic end products of digestion (Pethick et al., 1991).

The $EE_{resting}$ value for the non-working cow was found to be 30.38 MJ/d (table 1) which is equivalent to 0.42 $MJ.kgW^{0.75}$. ME_m can be derived from this value using the formula EE at maintenance (ME-RE/0.70). At RE=0, ME_m equals EE at maintenance. If ME intake increased by $\triangle$ME, an increment $\triangle$RE will be retained. Mount (1970) postulated that $\triangle RE/\triangle ME=0.70$, thus only 70% of an increase of ME above maintenance will be retained and the remainder will be lost as heat. From this relationship, ME_m for the swamp buffalo was found to be 0.37 $MJ.kgW^{0.75}/d$. which is 1.25 times basal metabolic rate (Brody, 1945). Using a mask method Sastradipradja (1965) found a similar value of 0.37 $MJ.kgW^{0.75}/d$ (87.7 $kcal.kgW^{0.75}/d$) for standing resting fasting buffaloes, a situation less than ME_m. The calculation of ME requirement for growth for our buffaloes was based on the regression relationship between RE and $ME/W^{0.75}$; all values were corrected free from exercise energies. Calculations from retained protein and fat demonstrated that 14.6 MJ ME/d was required for each kg increase of body mass.

Energy for work ($E_{exercise}$) calculated from $EE_{working}$ substracted by $EE_{resting}$ showed that the value for the 1 h working group was 9.56 MJ/d, while those for the 2 h and 3 h working groups were 20.0 and 25.86 MJ/d, respectively. The daily $EE_{working}$ values were equivalent to respectively 1.31, 1.76 and 1.99 times $EE_{resting}$. These values are within the range of 1.2 to

2.0 ME_m reported by Bakrie and Ma'sum (1993) for draught animals undertaking light to heavy work. Extrapolation of the $EE_{working}$ data to zero h work (resting) resulted in EE equal to 31.33 MJ which is greater than the $EE_{resting}$ of 30.38 MJ. The discrepancy of 0.95 MJ is attributed to a condition of alertness required for doing the physical activity associated with draught. The relatively low energy requirement for alertness supports the notion that the swamp buffalo is a quiet non-temperamental animal suitable for draught. The amount of EE (MJ/d) of working buffalo cows is dependent on body mass W (kg), draught force F (kN) and work duration t (h) according to equation: $EE_{working}=1.24W^{0.66}F^{0.35}t^{0.26}$ (r^2=0.97, SD=0.01), while $E_{exericise}$ (MJ) relates to F and t according to equation: $E_{exercise}=[0.003F^{1.43}t^{0.93}]/W^{0.09}$ (r^2=0.99, SD=0.005).

The breakdown of $E_{exercise}$ into its components following the Lawrence factorial approach requires the use of the coefficients a, b, and c. The original coefficients (Lawrence, 1985; Lawrence and Sibbard, 1990) were obtained from laboratory spirometric measurements which may preclude the heat increment associated with exercise remaining residual in the body post-exercise. Our $E_{exercise}$ data incorporated this heat increment and therefore alternative values for a, b and c are needed under the condition of the present study. For the calculations, the Lawrence equation is modified into a multiple regression form: $Y=aX_1+BX_2+CX_3$, where X_1=WD, $X_2=F\sin\alpha D$, $X_3=F\cos\alpha D$ and C=1/c. The data required for these calculations are presented in table 2. Thus coefficient a, which is the energy required to move 1 kg BW 1 m away, is 2.56 $\pm$0.029 J; b, which is the energy to move 1 kg load 1 m away is 5.2$\pm$0.027 J; and c, which is the mechanical efficiency factor, is 1/3.5793 (0.29). $E_{exercise}$ values for control, 1, 2 and 3 h work calculated according to the Lawrence factorial method were therefore found to be 0, 6.40, 13.91 and 18.01 MJ/d, corresponding to efficiencies to convert ME into $E_{exercise}$ to be 0, 16.09, 27.36 and 32.44%, repectively.

Protein requirement was estimated from the feeding trials and changes in body composition. The calculations required data on digestibility and the biological value of protein, and yielded a value of $2.51W^{0.75}$ g/d digestible protein for maintenance, whereas the requirement for work depended on the length of carrying out work (for loads 450 to 500 N). The relationship between duration of work (t, h) and digestible protein requirement (DP_{work}) follows the equation: $DP_{work}=[12.59e^{0.95t}]$g. Protein requirement for growth of the working swamp buffalo was calculated from daily gain in body weight, body composition, the biological value of protein and nitrogen loss through urine. For each kg increase of body mass, 181 g of protein is needed (protein content of the body is 18.1%). Assuming a biological value of protein to be 70%, digestible protein with feed intake should be 258 g (181/0.70). In addition, assuming endogenous nitrogen excretion in urine is $0.2W^{0.75}$g/d (Brody, 1945), then urinary protein would be $1.25W^{0.75}$g/d ($6.25\times0.2W^{0.75}$). Based on these figures, daily digestible protein requirement for the growing buffalo cow (DP_{growth}) would be :

$DP_{growth}=[(258+1.25W^{0.75})\Delta W\ kg/d]g$

Table 3 shows energy and protein requirements of 250 to 400 kg young swamp buffalo cows employed for light, moderate and heavy draught, to supply energy and protein equalling the maintenance requirements plus additional feed given on the basis of our best estimate of their energy and protein needs for growth and work performed. Further research to confirm extrapolated requirements is required.

Table 2. Measured components of work energies for the calculation of the modified constants a, b and c of the Lawrence equation $E_{exercise}{}^1=aWD+bLD+E_{pull}/c$ for traction on a flat surface by swamp buffalo cows

Exercise	$E_{exercise}{}^1$ (MJ)	WD (kg.km)	LD^2= ($F\sin\alpha$)D (kg.km)	$E_{pull}{}^2$= ($F\cos\alpha$)D (kg.km)
1 h daily	8.937	0.810	0.457	1.255
	9.049	0.924	0.505	1.141
	8.500	0.773	0.434	1.193
	8.886	0.884	0.500	1.123
2 h daily	19.209	2.022	0.933	2.564
	19.258	1.944	1.078	2.422
	19.276	1.977	0.945	2.598
	18.876	1.787	1.080	2.427
3 h daily	24.190	2.574	1.170	3.216
	25.484	2.712	1.399	3.143
	24.316	2.660	1.164	3.197
	24.743	2.520	1.381	3.101

[1] $E_{exercise}=EE_{working}-EE_{resting}$ ($E_{exercise}$=energy used for the physical exercise by the animal; $EE_{working}$=daily energy expenditure of the animal during a working day; $EE_{resting}$=daily EE of the animal during a day of rest); W=liveweight (kg); D=distance travelled (km); L=load carried (kg)=$F\sin\alpha$; F=measured draught force (kg); E_{pull}=work done to pull a load (kJ)=$F\cos\alpha$ (kg)$\times$D (km).

[2] α=traction angle; the value in these trials varied between 20-23°.

By least square method values were found for a=2.56 J/kgW.m, b=5.2 J/kgL.m and c=0.29 (29%).

An example of ration composition offered to 300 kg buffalo cows undertaking draught and allowing them to grow 0 to 0.5 kg body mass per day would be:

a) for draught 1 h/d: 100% king grass;
b) for draught 2 h/d: 75% king grass plus 25% Glyricidia leaves;
c) for draught 3 h/d: 50% king grass, 30% Glyricidia leaves plus 20% rice bran.

Table 3. Daily metabolizable energy and digestible protein requirements of draught swamp buffalo cows age 2 to 4 years (W: 250 to 400 kg) undertaking traction work F of 450 to 500 Newton (N) for a period t (h) up to 3 h daily*

W	⊿W	No Work		Light Work		Moderate Work		Heavy Work	
		ME	DP	ME	DP	ME	DP	ME	DP
kg	kg/d	MJ/d	g/d	MJ/d	g/d	MJ/d	g/d	MJ/d	g/d
250	0	26.4	158	36.2	190	45.0	242	53.5	375
	0.25	30.1	242	39.9	274	48.7	326	57.2	459
	0.50	33.7	326	45.5	358	52.4	400	60.9	543
300	0	30.3	181	40.1	213	48.9	265	57.4	398
	0.25	33.9	268	43.8	300	52.6	352	61.1	485
	0.50	37.6	355	47.5	387	56.3	439	64.8	572
350	0	34.0	203	43.8	235	52.6	287	61.1	420
	0.25	37.6	292	47.5	324	56.3	376	64.8	509
	0.50	41.3	382	51.1	414	60.0	466	68.5	599
400	0	37.6	225	47.4	257	56.2	309	64.7	442
	0.25	41.2	317	51.1	349	59.9	401	68.4	534
	0.50	44.9	409	54.8	441	63.6	467	72.1	626

W=body mass; ⊿W=daily increase in body mass; ME=metabolizable energy, daily requirement; DP=digestible protein, daily requirement.

* The calculations of ME and DP requirements were based on the following assumptions: $ME_{resting}=0.42$ MJ/d.kg$^{0.75}$; $EE_{working}=1.24W^{0.66}F^{0.35}t^{0.26}$ MJ/d (F in kN; ME increments above resting are 9.80, 18.60 and 27.10 MJ for light, moderate and heavy daily work, respectively); ME_{growth} is 14.6 MJ/d for each kg increase of body mass; $DP_{maintenance}=2.51W^{0.75}$ g/d; $DP_{work}=[12.59e^{0.95t}]$ g; $DP_{growth}=[(258+1.25W^{0.75})\varDelta Wkg]$ g/d.

In conclusion, the body density method to measure *in vivo* body composition applied to swamp buffaloes is proven to be very useful and appropriate because wading in water is a natural behaviour of these animals. Combined with material balance and exercise trials in the field, energy and nutrient requirements under the prevailing environmental and country condition could readily be estimated.

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