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# Effect of maternal supplementation of seaweed byproducts on nutrient intake and digestibility in advanced pregnant murrah buffaloes

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#### Abstract

The present study was conducted in Livestock Research Centre, National Dairy Research Institute, Karnal Haryana to evaluate the effect of maternal supplementation of seaweed by products (SWBP) on the nutrient intake and digestibility in advanced pregnant Murrah buffaloes. Twelve Murrah buffaloes (~60d prepartum) were assigned to three groups (Control,  $T_1$  and  $T_2$ ) with 4 animals each. All the buffaloes were fed basal diet (wheat straw, green fodder and concentrate mixture) as per ICAR, 2013 requirements with additional supplementation of SWBP-1 (*Kappaphycus alverazii and Gracilaria salicornia*) in  $T_1$  and SWBP-2 (*Kappaphycus alverazii, Gracilaria salicornia and Turbinaria conoides*) in T<sub>2</sub> at 2.5% of concentrate mixture. Supplementation period started from 60d prepartum and continued till calving. A digestibility trial of 7 days was also conducted. Proximate analysis of seaweeds showed that *T. conoides* had highest CP (%) (7.84) followed by *K. alverazii* (5.87) and *G. salicornia* (4.20). Highest TA (%) was found in *G. salicornia* (77.97) and in other two seaweeds the values were comparable. The results revealed that there was no significant (*P*>0.05) difference in DMI (kg/d), CPI (kg/d) and TDNI (kg/d) among the three groups. Digestibility of nutrients (%) was not affected due to supplementation of SWBP. From the present study it can be concluded that maternal supplementation of SWBP did not affect nutrient intake and digestibility in advanced pregnant Murrah buffaloes.

Keywords: murrah buffaloes, nutrient intake, seaweed byproducts, SWBP-1, SWBP-2

#### Introduction

India has more than 8100 km of coastline that harbours a wide variety of seaweed species. Oza and Zaidi (2001) <sup>[21]</sup> reported that there are 844 species of seaweeds with 434, 194, and 216 species of red, brown, and green seaweeds, respectively. Coasts of Gujarat and Tamil Nadu are known for their relative abundance of seaweeds (Jha et al., 2009)<sup>[3]</sup> and are home to 366 seaweed species that account for nearly half of India's total seaweed diversity (Ganesan, 2017) <sup>[9]</sup>. Around coastal areas of Tamil Nadu, a particular type of seaweed (*Gracilaria edulis*) is being used since decades for making gruel. The composition of seaweeds depends on the species, season, habitat, and prevailing proximate environmental conditions such as desiccation, air and water temperature, light intensity, and nutrient concentrations (Marsham et al., 2007) <sup>[15]</sup>. Seaweeds are rich in soluble dietary fibres, proteins, minerals, vitamins, antioxidants, phytochemicals, and polyunsaturated fatty acids and have a low caloric value (Mohamed, 2012)<sup>[16]</sup>. The bioactive substances like polysaccharides, proteins, lipids and polyphenols present in seaweeds have antibacterial, antiviral and antifungal properties (Kumar et al., 2008)<sup>[14]</sup>. The compounds present in seaweeds exhibit higher water holding capacity than cellulosic (insoluble) fibres. Soluble dietary fibres demonstrate the ability to increase viscosity, form gels and/or act as emulsifiers (Elleuch et al., 2011)<sup>[6]</sup>. Consumption of seaweed products has recently increased, approximately 15-20 edible algae species being commonly marketed for consumption (Peinado et al., 2014) <sup>[21]</sup>. In Asia, seaweeds are consumed as a vegetable and on an average, the Japanese eat 1.6 kg seaweed per person per year (Fluerence, 1999)<sup>[8]</sup>. Seaweeds have been proved to exhibit potent medicinal effects against cancer, allergy, diabetes, oxidative stress, inflammation, thrombosis, obesity, lipidemia, hypertensive and other degenerative ailments (Mohamed, 2012) [16]. Commercially, seaweeds are used as food, ingredients in fertilizers and cosmetics, and in production of agar and alginate (Chan and Phang, 2006)<sup>[4]</sup>. Green algae contain sulphated galactans and xylans, brown algae are rich in alginic acid, fucoidan (sulphated fucose), laminarin ( $\beta$ -1, 3 glucan) and sargassan whereas red algae are rich in agars, carrageenans, floridean starch (amylopectin-like glucan), water-soluble sulphated galactan, as well as porphyrin located as

mucopolysaccharides in the intercellular spaces (Murata and Nakazoe, 2001)<sup>[18]</sup>. Alginic acid present in seaweeds reduces the concentration of cholesterol, prevents the absorption of toxic substances and helps in maintenance of animal and human health by playing an important role as dietary fibre (Nishide and Uchida, 2003)<sup>[19]</sup>. Global production of seaweeds has been estimated at 30.1 million wet tons in 2016, out of which the culture sector contributes 95% (Ferdouse et al., 2018)<sup>[7]</sup>. The annual standing crop of marine algae of India has been estimated as 3,01,646 tonnes (FAO, 2016). Moreover, seaweeds offer additional advantages, as their cultivation does not compete with terrestrial agriculture, do not need fresh water, and the aquatic photosynthesis contribute to reduce CO<sub>2</sub> levels. In India, agar and alginate are produced from seaweeds harvested from wild stocks whereas carrageenan is obtained from cultivated Kappaphycus alvarezii (Doty) (Ganesan et al., 2019)<sup>[10]</sup>. Use of seaweeds in animal feeding could also help to alleviate the environmental pollution caused by management of seaweeds in coastal zones. On the other hand, seaweed farming is known to render environmental benefits by recycling nutrients and preventing eutrophication. Thus, there is a huge potential of utilizing these wonder plants of the sea for livestock feeding allowing for improved animal health and performance.

# **Material and Method**

This study was undertaken at the Animal Nutrition Division, ICAR-NDRI, Karnal to evaluate combinations of seaweed byproducts as an agent for maternal programming. Supplementation of seaweed by-products was carried out in pregnant Murrah buffaloes and its effect was studied on their neonatal calves. The details of the experimental procedures employed and methods adopted during the course of study are described briefly here. The experimental protocols carried out in the study were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of Ministry of Environment, Forests and Climate Change, Government of India, New Delhi. Procurement of seaweed byproducts were done from Aquagri Processing Private Limited, New Delhi under CSIR sponsored project entitled, "Kappaphycus alvarezii and red seaweed-based formulations for improving productivity and health of dairy animals and poultry". Seaweed byproducts of different species were used to make two combinations for supplementation in the diet of pregnant Murrah buffaloes.

Pooled samples were analyzed for chemical composition according to standard methods of AOAC (2005) [11]. Fiber fractions were assayed using by procedures of Van Soest et al. (1991)<sup>[26]</sup>. Acid detergent lignin was recovered from ADF by solubilizing cellulose with 72% (w/w) sulphuric acid (Van Soest et al., 1991)<sup>[26]</sup>. Twelve Murrah buffaloes were taken divided in 3 groups, Control (C), T1 and T2 with 4 animals in each group. Control group was fed with basal diet only, T1 with basal diet plus SWBP-1 @2.5% of concentrate mixture and T2 group with basal diet plus SWBP-2 @2.5% of concentrate mixture. All the experimental animals were offered weighed quantity of concentrate mixture once at 09:30 AM and then at 3:30 PM, wheat straw at 9:30 AM and green fodder at 11:00 AM to meet their requirements for maintenance and also for pregnancy (ICAR, 2013)<sup>[12]</sup>. The amount of feed offered and refusals from all the animals was weighed daily and sampled twice in a week to assess average DM intake throughout the experiment. Clean drinking water was made available daily at 10:00 AM and 04:00 PM. A digestion trial of 7 days collection period was conducted during the experimental period. During the collection period all the animals were offered accurately measured amount of concentrate mixture, jowar fodder and wheat straw and their residues were also measured. Representative samples of feed offered, residues and faeces voided were collected at 24h interval for further sampling in laboratory for analysis and to know the digestibility of nutrients. Intake and digestibility of DM, OM, NDF and ADF were calculated by subtracting faecal loss of these nutrients from intake of these nutrients during digestion trial in each experimental period. Data were analyzed using the general linear models (GLM) procedure of SPSS 16.0 computer package.

#### **Results and Discussion**

# Nutrient composition (% DM basis) used during study trial

Nutrient composition of diet is presented in Table 1. Values for DM, OM, TA, CP, EE, NDF and ADF in sorghum fodder were 31.58%, 89.96%, 10.04%, 6.59%, 1.8%, 61.85% and 28.64% whereas the concentrate mixture contained 89.97% DM, 92.72% OM, 7.28% TA, 18.03% CP, 4.29% EE, 26.28% NDF and 10.84% ADF. These values for wheat straw were 90.17% DM, 89.22% OM, 10.78% TA, 3.07% CP, 1.17% EE, 75.81% NDF and 50.92% ADF.

Parameter	Wheat Straw	Green fodder (Sorghum)	Concentrate mixture
DM	90.17±0.13	31.58±1.11	89.97±0.54
OM	89.22±0.06	89.96±0.46	92.72±0.07
ТА	10.78±0.06	$10.04 \pm 0.46$	7.28±0.07
СР	3.07±0.05	6.59±0.07	18.03±0.07
EE	1.17±0.04	1.80±0.09	4.29±0.06
NDF	75.81±0.77	61.85±2.59	26.28±0.21
ADF	50.92±0.91	28.64±0.12	$10.84 \pm 0.18$
Cellulose	39.78±0.61	20.86±0.39	3.11±0.12
Hemicellulose	24.89±1.21	33.21±2.65	15.4±0.36
Lignin	6.33±0.16	4.38±0.11	1.04±0.10
TDN	46.03±0.29	54.87±0.86	75.38±0.27
MP	1.55±0.07	4.05±0.03	11.20±0.05
ME (Mcal/kg)	1.51±0.01	1.94±0.04	2.99±0.01

Table 1: Nutrient composition	n (% DM basis)	used during study trial
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Chemical composition (% DM basis) of seaweed byproducts and their formulations

The detailed chemical composition of seaweed byproducts

and their formulations is presented in Table 2. In *Gracilaria* salicornia, the percentage of DM (95.84), TA (77.97) and AIA (32.46) was highest whereas the percentage of OM

(22.03), CP (4.20) and EE (0.30) was lowest. The percentage of DM, OM, CP, EE, TA, AIA in *Kappaphycus alverazii* and *Turbinaria conoides* ranged from 88.83 to 91.76, 62.12 to 66.81, 5.87 to 7.84, 0.49 to 0.63, 33.19 to 37.88 and 8.80 to 9.53 respectively. Out of the two seaweed byproducts formulations, SWBP-1 had higher percentage of DM (93.80), TA (57.91) and AIA (20.62) whereas SWBP-2 contained higher percentage of OM (50.55) and CP (5.97). Both the seaweed byproduct formulations had similar percentage of EE (0.47). Out of three, red seaweed *Gracilaria salicornia* had the highest NDF and ADF content (35.11 and 15.92% respectively) followed by red seaweed *Kappahycus alverazii* (28.75 and 14.04% respectively) and lowest in *Turbinaria* 

conoides (25.47 and 10.23% respectively).

Composition of seaweed byproducts found in this study are in accordance with values found by Sharma *et al.* (2020) <sup>[223]</sup> except that they reported higher values for TA and EE whereas lower values for OM in the seaweed product. Munde (2019) <sup>[17]</sup> reported similar chemical composition of *Kappaphycus* and *Gracilaria* except that the TA and AIA content of *Gracilaria* found in our study were higher. The composition of seaweeds is highly variable therefore the deviation in chemical composition of seaweed products found by various researchers might be due to varying species, season, habitat and microclimate such as water temperature, light intensity and nutrient concentrations.

Table 2: Chemical composition (% DM basis) of seaweed byproducts and the	eir formulations
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Ingredient	DM	ОМ	ТА	СР	EE	NDF	ADF
KA	91.76±0.42	62.12±0.84	37.88±0.84	5.87±0.06	0.63±0.02	28.75±0.09	14.04±0.13
GS	95.84±0.53	22.03±0.51	77.97±0.51	4.20±0.06	0.30±0.02	35.11±0.05	15.92±0.17
TC	88.83±0.66	66.81±0.78	33.19±0.78	7.84±0.04	0.49±0.03	25.47±0.11	10.23±0.10
SWBP-1	93.80±0.86	42.09±0.64	57.91±0.64	5.04±0.06	0.47±0.02	31.9±0.02	14.92±0.08
SWBP-2	92.15±0.49	50.55±0.33	49.45±0.33	5.97±0.08	0.47±0.05	29.71±0.07	13.22±0.06

# Body weight of advanced pregnant Murrah buffaloes during study trial

The data pertaining to body weight of advanced pregnant Murrah buffaloes is presented in Table 3. The initial body weight at 60d pre-partum was similar in all three groups and ranged from 628.9 kg to 656.5 kg with a period mean of 640.9 kg. The body weight of animals remained statistically similar among the treatment groups throughout the study. These results indicate that there was no influence of supplementation of seaweed byproducts on body weight of animals, however, an increase in body weight was found in all the groups as the parturition approached which maybe attributable to weight of calf and products of gestation.

Table 3: Body weight of the animals measured during study trial.

Days prepartum	Control	$T_1$	$T_2$	
60d	637.2±28.87	656.5±16.14	628.9±37.28	
45d	648.9±28.86	669.7±17.17	644.5±38.60	
30d	663.4±29.15	685.8±18.23	662.1±40.15	
15d	680.7±28.91	703.7±19.31	681.9±41.29	
7d	699.8±28.50	724.0±20.46	702.7±41.75	
Average	666.0±12.56	687.9±8.94	664.0±16.94	

## Nutrient intake and digestibility in advanced pregnant Murrah buffaloes during digestibility trial

Average values of nutrient intake and digestibility during the digestibility trial have been given in Table 4. The average DM intake in C, T<sub>1</sub> and T<sub>2</sub> were 11.21, 11.32 and 10.89 kg/d respectively which when expressed as % of ICAR requirement was 72.62, 71.96 and 70.62 in C,  $T_1$  and  $T_2$ respectively. Intake of CP in all the groups was found to be 0.94 kg/d whereas DCP intake varied from 0.55 kg/d in C and  $T_1$  groups to 0.57 kg/d in  $T_2$  group. CP intake expressed in terms of ICAR requirement was 99.55%, 98.38% and 99.46% in C, T<sub>1</sub> and T<sub>2</sub> groups respectively. The intake of TDN ranged from 6.11 to 6.25 kg/d in different groups which was 105.84% to 107.24% of ICAR requirement. The mean values of DM digestibility were 64.58%, 64.97% and 65.13% in C, T<sub>1</sub> and T<sub>2</sub> groups respectively. OM digestibility in different groups ranged from 66.57 to 67.62% whereas digestibility of CP varied from 57.91 to 60.08%. Digestibility of EE was 71.62% in C, 72.79% in T<sub>1</sub> and 73.48% in T<sub>2</sub> group whereas

for NDF, it ranged from 57.48 to 58.78% in all groups. ADF digestibility was 49.21%, 49.99% and 51.27% in C,  $T_1$  and  $T_2$  group whereas in the respective groups, digestibility values for TDN were 54.94, 55.16 and 55.8%.

Statistical analysis of data in Table 4 revealed that nutrient intake and digestibility among treatment groups did not change due to supplementation of seaweed byproducts. It is from the data that seaweed evident byproducts supplementation up to 2.5% of concentrate mixture did not affect the palatability of diet, hence the DM intake remained similar in all the groups. Also, the digestibility of proximate principles and cell wall constituents was not influenced by seaweed byproducts supplementation, leading to similar TDN values in different groups. Antaya et al. (2019)<sup>[2]</sup> reported a quadratic increase in DMI with increased level of seaweed Ascophyllum nodosum in the diet. El-Din et al. (2008)<sup>[5]</sup> reported that DM, TDN and DCP intake were significantly (P < 0.05) higher by calves fed kelp meal than that by control ration and buffer supplemented ration during all experimental periods. The intake as DM, TDN and DCP increased by 6.80, 11.60 and 15.15% during the whole experimental period by calves fed kelp meal compared with those fed control diet, respectively. Our results corroborate with the findings of Sharma et al. (2020) [23] and Singh et al. (2016) [24] who reported no change in DMI of cows fed with seaweed powder. Spiers et al. (2004)<sup>[25]</sup> reported that feed intake of growing steers did not change when seaweed was supplemented in their diet. Inclusion of brown seaweed at 0.25 and 0.5% DM in the diet of dairy cattle did not affect overall DMI (Pompeu et al., 2011)<sup>[22]</sup>. In a study by Abdoun et al. (2013)<sup>[1]</sup>, seaweed inclusion in the diet of lambs had no effect on daily feed intake. Contrary to our findings, Cabrita et al. (2016)<sup>[3]</sup> reported a decrease in DM intake by 24 and 25% when alfalfa hay was supplemented with Ulva rigida and Gracilaria vermiculophyla respectively. This might be due to an inclusion level of seaweeds at 20% of diet which is very high as compared to our study.

Supplementation of seaweed byproducts had no effect on digestibility of DM, OM, CP, EE, NDF, ADF and NFC. This indicates that seaweed byproducts when fed at 2.5% of concentrate mixture did not impact nutrient digestibility in animals. Our results coincide with the findings of Antaya *et* 

*al.* (2019) <sup>[2]</sup> who reported no change in digestibility of DM, OM, NDF and ADF when diet was supplemented with kelp meal. Similarly, when pigs were supplemented with *Laminaria* seaweed extract, there was no effect on nutrient digestibility (Reilly *et al.*, 2008). Munde (2019) <sup>[17]</sup> found no change in nutrient digestibility in crossbred cattle when

seaweed byproducts were supplemented in their diet. In contradiction, Cabrita *et al.* (2016) <sup>[3]</sup> reported that digestibility of diet containing seaweeds were lower by 4 to 6% than control diet containing no seaweed. In their study, they used 20% inclusion level of seaweeds which may be the main reason for lower digestibility of nutrients.

'able 4: Nutrient intake and digestibility in advanced	l pregnant Murrah buffaloes	during digestibility trial
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Nutrient intake	Control	$T_1$	$T_2$	P- value	SEM		
DMI (kg/d)	11.21±0.217	$11.32 \pm 0.078$	10.89±0.187	0.161	0.094		
DM requirement (kg/d)	15.44±0.263	15.76±0.165	15.44±0.303	0.528	0.137		
DMI% of ICAR	72.62±1.012	$71.96 \pm 0.807$	70.62±0.451	0.228	0.464		
CPI (kg/d)	0.94±0.016	$0.94 \pm 0.006$	0.94±0.015	0.995	0.007		
CP requirement (kg/d)	$0.94 \pm 0.008$	$0.95 \pm 0.005$	0.94±0.103	0.513	0.004		
CPI% of ICAR	99.55±1.165	98.38±0.523	99.46±0.48	0.461	0.434		
DCPI (kg/d)	0.55±0.012	0.55±0.01	0.57±0.021	0.495	0.008		
TDNI (kg/d)	6.17±0.151	6.25±0.09	6.11±0.198	0.791	0.083		
TDN requirement (kg/d)	5.69±0.07	5.75±0.04	5.83±0.09	0.314	0.037		
TDNI% of ICAR	107.24±2.302	107.24±1.83	105.84±2.429	0.875	1.229		
Nutrient digestibility %							
DM	64.58±0.672	64.97±0.83	65.13±1.113	0.909	0.504		
OM	66.57±0.61	66.95±0.779	67.62±1.135	0.705	0.491		
CP	57.91±0.646	58.39±1.028	60.08±1.541	0.389	0.647		
EE	71.62±0.839	$72.79 \pm 0.605$	73.48±0.759	0.217	0.422		
NDF	57.48±0.814	58.58±1.098	58.78±1.311	0.684	0.629		
ADF	49.21±1.068	49.99±1.206	51.37±1.37	0.483	0.705		
NFC	92.63±0.253	90.56±1.204	91.86±0.933	0.295	0.553		
TDN%	54.94±0.568	55.16±0.713	55.8±1.041	0.745	0.451		

## Conclusions

Thus, it can be concluded that supplementation of tropical seaweed byproducts at 2.5% of concentrate mixture in the diet of advanced pregnant Murrah buffaloes did not affect nutrient intake and digestibility.

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