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Effect of Variable Protein and Energy Diet on Purine Derivatives Excretion and Quantitative Microbial Protein Production in Crossbred and Indigenous Calves

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A B S T R A C T

Study was conducted to evaluate the effect of enhanced level of nutrition on nutrient intake and microbial protein (MBP) production of two genotypes; Deoni and HF crossbred calves. Treatment groups of Deoni (DTG) and HF crossbred (XTG) were fed 30% more protein and energy as compared to their respective control groups (CGs). Dry matter intake (DMI) of DTG was reduced by 18% in comparison to DCG. Crude protein (CP) intake in DTG was 312 g/d and was higher by 14% compared to DCG. Whereas, CP intake in XTG was 38% more compared to XCG. Gross energy (GE) intake by DTG was 8.13 Mcal/d compared to 9.70 Mcal/d of DCG. The MBP production in XCG, XTG, DCG and DTG was 239, 229, 143 and 142 g/d, respectively. Quantitative availability of MBP was constant and related to size of the calf. This indicated that the DMI in Deoni calves was regulated by the nutrient density of diet indicating towards the low level of phenotypic plasticity of digestive system in Deoni calves providing less scope for productive improvement by nutritional intervention compared to HF crossbred calves.

Introduction

Nutrition is the primary entity to be look after for better body growth. Faster growth entails more amounts of nutrients input through the diet. Often growth slumps immediately after weaning due to shift in nutrition from high density nutrient diet to roughage-based diet with supplements. And also, nutrition is all too often neglected between weaning and 9 months of age. Weaning to 9 months of age is a critical time in the life of a calf and is a time of higher nutrient requirements. As rumen capacity is limited during this period, a diet high in protein and energy content should be provided. In absence of adequate nutrition, the average daily weight gain (ADG) of calves would be less than genetic potential and fails to obtain required body weight as per the age. Insufficient growth rates result in an older age at first calving (Van Amburgh *et al.*, 2008) which ultimately affects the overall productive life of the animal. Diet with CP content of 17 to 18% (DM basis) is recommended for calves less than 250 kg BW (Kertz *et al.,* 1987). Brosh *et al.,* (2000) have noted higher weight gain in male HF calves fed high protein diet (14.6%) in comparison to feeding medium (12.6%) or low (10.6%) CP diets. Whereas, Lohakare *et al.,* (2006) found a diet with 12.6% CP can provide adequate digestible protein at the intestine for the growth. Purine derivative (PD) excretion in the urine has proved to be a useful index of microbial protein flow to the small intestine of ruminants (Chen *et al.,* 1990; Balcells *et al.,* 1991). The efficiency of microbial nitrogen supply (EMNS), in terms of g microbial N/kg varies at different level of intake (Liu *et al.,* 2000). Researchers have suggested that the urinary excretion of PD could be used as a predictor of the MBP supply in intact animals (Arndt *et al.,* 2015; Wang *et al.,* 2015).The purpose of this investigation was to study the effects of increased protein and energy level in the diet on intake and microbial protein production pattern.

Materials and Methods

Location of study

Experiments were conducted at Livestock Research Centre, Southern Regional Station, ICAR-NDRI, Bengaluru. The latitude (Φ), longitude (λ) and elevation of the experimental place is 12.947014°N (12° 56' 49.2504" N), 77.607679 (77° 36' 27.6444" E) and 921 m from mean sea level, respectively. The climate here is tropical. The summers are much rainier than the winters in Bengaluru. This climate is considered to be Aw according to the Köppen-Geiger climate classification. The temperature here averages 23.6 °C. In a year, the average rainfall is 831 mm. The driest month is January, with 1 mm of rain. The greatest amount of precipitation occurs in September, with an average of 182 mm. April is the warmest month of the year. The temperature in April averages 27.1 °C. The lowest average temperatures in the year occur in December, when it is around 20.7 °C. There is a difference of 181 mm of precipitation between the driest and wettest months. The variation in temperatures throughout the year is 6.4 °C (https://en.climate-data.org/location/4562/). The experiment was conducted between September 2016 and February 2017.

Experimental design and management of Animals

The experimental design was a randomized complete block design with four treatments and five replications. Weaned female calves of Deoni and HF crosses at the age of 3 months were selected ten each for the study and randomly distributed into 4 equal groups; 1) Deoni Control Group (DCG), 2) Crossbred Control Group (XCG), 3) Deoni Treatment Group (DTG) and 4) Crossbred Treatment Group (XTG).The mean birth weights of Deoni and HF cross calves were 19.90±2.02 and 29.40±4.67 kg respectively. The body weights (BW) at the start of experiment were 61.90±4.45 and 64.70±6.18 kg for Deoni and Crossbred (CB) calves respectively. Calves were housed in a well-ventilated shed having arrangement for individual feeding, well maintained drainage channels and covered with laterite clay tiles on a 15 ft wall with 45° inclination on 6 ft stone pillars. All the experimental animals were kept under confinement in individual stalls under tail to tail systemand were provided free access to water. Animals were dewormed with generic product ‘Fenbendazole’ (Panacur® Vet, M/s Intervet India Pvt. Ltd.) prior to starting of experiment.

Feeding of animals

Based on the NRC (2001) recommendation and earlier records of growth rate, nutrient requirement for the CG of Deoni and crossbred calves was calculated. TG of Deoni and crossbred weaned calves were provided 30% enhanced protein and energy over, respective CGs. Calves were fed mixed grasses consisted of Para grass (*Brachiariamutica*), Hybrid Napier (*Pennisetumglaucum* X *P. purpureum*), Maize (*Zea mays*) and Guinea grass (*Megathyrsus maximus*) as green roughage sources and CS pellet (M/s Karnataka Milk Federation [KMF], Bangalore). CG and TG of Deoni and Crossbred weaned calves were fed, respective diets for a period of 6 months. Weighed quantities of CS were fed once daily at 8 AM. Mixed green grasses were fed at 10:00 AM.The ingredient composition and nutritive values of diets are presented in Table 1.

Feeding trial and sample collection

CG and TG of Deoni and Crossbred weaned calves were fed, respective diets for a period of 6 months. Digestibility trial was conducted after a preliminary period of feeding for 5 months. Trial was consisted of 5 d collection period. Feeding schedule of the calves during the trial period was also maintained as described above. Diet offered as well as refused (orts) was weighed daily. Representative samples of of each feedstuff offered, and residues were collected every day for evaluating the DM and for further laboratory analysis. Faecal sampling was done every day at 9:00 AM. The total quantity of faeces voided by each animal during 24 h was recorded, thoroughly mixed with gloved hands and composite sample of 150 to 200 g faeces was taken separately for each animal in clean dry polythene bags for further sub-sampling. Sub-sample of 1/100 of total faeces was taken for DM estimation i.e., about 50 to 60 g. Dried samples of feed offered, orts, and feces were pooled for 5 d. Another sub-sample of 1/400 of total faeces was preserved with known quantities of 10% H_2SO_4 ($^V/_V$) in pre-weighed glass bottles for nitrogen estimation.

Urine collection

Spot urine sample was collected 2-3 times a day for 3-4 d at different time. 20 ml of sample was collected from each calf every time and were filtered using four layers of cheesecloth following the method described by Balcells *et al.*, (1992) and mixed with equal quantity of dilute sulphuric acid (2% H_2SO_4) to avoid volatilization of ammonia and stored at -20°C. Finally, one sample of each calf is pooled from all the samples of the same animal and were stored at -20°C and preserved till further analysis.

Chemical analysis

Feed (offered and residue) and faecal samples collected during the digestibility trial were analysed for proximate principles and cell wall constituents (CWC) as per AOAC (2012). Proximate principles and CWC was determined as difference between total intake and faecal output.

Microbial protein estimation

Microbial nitrogen (MBN) production was estimated from the spot urine samples using purine derivatives (PD) and creatinine as markers in the urine (Chen *et al.*, 1995). Urine samples were thawed, and 20 ml aliquots were taken, centrifuged and filtered through a Millipore filter of 0.2 µm pore size (M/s Sigma Aldrich, Cat no. F-1387). One ml of the filtrate was taken and diluted 10-fold with HPLC grade water after adjusting the pH to 7.0 using 0.01 N NaOH and 0.01 N H_2SO_4. 20 µl of processed sample was injected into HPLC. Allantoin, creatinine and uric acid in the urine were estimated using HPLC method (Aswin and Srinivas, 2015). Standard solutions of allantoin (M/s Sigma Aldrich, Cat no. 93791), creatinine (M/s Sigma Aldrich, Cat no. C4255) and uric acid (M/s Sigma Aldrich, Cat no. U0081) each containing

50µg/ml ($^w/_v$) of respective standards was separately prepared in HPLC grade water. HPLC (M/s Waters India Pvt., Ltd.) was run in isocratic condition using C-18 reverse-phase column (M/s Waters India Pvt., Ltd., and 4.5 × 260 mm I.D., 5µm) with 10mM potassium dihydrogen phosphate (pH 4.7) as mobile phase. Flow rate was fixed at 1 ml/minute and reading was taken at 220 nm wavelength (Model 2489 UV/visible detector, M/s Waters India Pvt., Ltd.,). Peaks were standardized for allantoin, uric acid and creatinine by injecting 20 µl of respective standard.

Excretion of total PD was calculated as below (Chen *et al.,* 1995):

PDC index: It was calculated as the ratio of the concentrations of PD (mM/L) to creatinine (mM/L) times the metabolic body weight (MBW)

PDC Index = [(PD mM/L)/ (Creatinine mM/L)] *MBW

The following equation was used to determine the quantitative relationship between absorption of purines and excretion of PD (Y mM/d) in urine.

$Y = 83X+0.296 \text{ kg } W^{0.75})$.

Where $W^{0.75}$ represents the MBW (kg) of the experimental calves. The slope of 0.83 in equation represented the recovery of absorbed purines as PD in urine and X was PDC index.

In crossbred cattle, the endogenous contribution was taken as a constant at $0.296W^{0.75}$ per day (Singh *et al.,* 2007; Pimpa *et al.,* 2001; Chen and Orskov, 2003). Thus, the daily purine absorption was back-calculated as

$X = (Y-0.296 \times W^{0.75}) \div 0.83$.

Intestinal flow of MBN was calculated from the excretion of PD as

$(X \times 70)/(0.83 \times 0.116 \times 1000)$.

Where, the digestibility of microbial PD was 0.83, N concentration in purine was 70 % and, 0.116 was the ratio between N in PD to total N in mixed rumen microbes.

Efficiency of MBN production was expressed as g N kg^{-1} of OM digested in the rumen (DOMR) by multiplying digestible OM by 0.65.

Statistical analysis

Statistical analysis of data was performed using statistical package for social science (SPSS, ver.20.0 M/s IBM India Pvt. Ltd.). The data were analysed in a completely randomized design and significant variation between means was denoted by different superscripts. Pairwise comparison between group means was tested by Duncan multiple range test (DMRT). Significant difference between groups was expressed at probability (P) levels ranging from < 0.10 to 0.01 and p value of < 0.10 was demarcated using '•' where as '*' and '**' were used to denote P value of < 0.05 and 0.01, respectively and validated against null hypothesis (H_0).

Results and Discussion

Chemical composition of basal diet and supplement

The chemical composition analysis of the experimental feeds items constituting treatment diets is presented in Table 1. DM and CP content of green grass which was mixture of non-legume grasses was 19.72% and 5.10%, respectively. The average CP content of the CS was 16%. Density of CP, NFC and energy in GNJ supplement was 63%, 74% and 10% higher than CS. The combination of GNC and Jaggery were supplemented to enhance intake of protein and energy by the calves.

Feed and nutrient intake

Nutrient intake from the total diet in different groups is given in Table 2. DMI from total diet was significantly less in DTG ($P \leq 0.01$) compared to DCG, XCG and XTG where later groups were statistically ($P = 0.08$) comparable. Similarly, OM and TCHO was also significantly less in DTG ($P \leq 0.01$) but, comparable between the crossbred groups. CP intake was significantly low in DCG ($P \leq 0.01$). Total OM ($P \leq 0.01$), EE ($P \leq 0.01$) and TA ($P \leq 0.01$) intake in XTG was higher than XCG. GE intake was significantly different among all the groups with DTG having the lowest value of 8.13 MCal/d whereas, XTG the intake was found highest (13.73 MCal/d) (Fig. 1)

Microbial Protein Production

Excretion of PD in urine and their index, MBP production with efficiency in different groups is presented in Table 3. Urinary allantoin excretion was significantly ($P \leq 0.01$) higher in TGs compared to respective CGs. Total PD was significantly low ($P \leq 0.01$) in DCG whereas it was comparable in XCG, XTG and DTG ($P = 0.051$). Similar was the case found regarding daily PD excretion with significant ($P \leq 0.01$) high value for HF crossbred calves. The MBP production in XCG, XTG, DCG and DTG was 239, 229, 143 and 142 g/d, respectively. Daily MBP production was significantly ($P \leq 0.01$) high in HF crossbred calves compared to Deoni animals. MBP production observed in XCG and XTG were 239 and 229 g/d and comparable to adult crossbred cows. The efficiency of MBP production in relation to DOMI and ME intake was found insignificant ($P = 0.15$) among the groups (Fig. 2).

Chemical composition of basal diet and supplement

DM and CP content showed that the green grass fed was matured and leafy with moderately tender stems. CP content of left-over green fodder was lesser than offered whereas NDF content was higher thus, indicated the selective intake of animals by taking more tender leafy parts and shoots but leaving behind the comparatively hard and fibrous parts like thickened stems with nodes and internodes (Antongiovanni and Sargentini, 1991). The CP content of the concentrate was16% which is considered average as according to Paulson (2009) the best CP% for dairy animals is 11 to 12%, 13 to 14% and 16 to 17%, respectively in low, medium and high protein CS based on health and environment.

Excess protein in the ration may have detrimental effect on kidney and body extremities e.g., hooves, tail etc.

Feed and nutrient intake

DMI of DTG was reduced by 18% in comparison to DCG. In-spite of less DMI, CP intake in DTG was higher by 14% compared to DCG because of higher protein in GNJ (26%) than pelleted CS (16%). In case of Deoni calves, OM, TA and TCHO intake were less in DTG and more in DCG while contrary was true for their EE intake. Although GNJ was rich in CP and GE (4 Mcal/kg), GE intake by Deoni calves of TG (8.13 vs. 9.70 Mcal/d) was 20% lesser ($P \leq 0.01$) than CG. This indicated that the DMI in Deoni calves was regulated by the satiety of CP intake. Diet composition has been suggested to have a large impact on satiety and satiation. It is generally believed that the major macronutrients differ in their effects, with protein having a greater effect than carbohydrate, which has a greater effect than fat (Hall *et al.,* 2012). Singh and Srinivas

(2016) observed 50% lesser DMI with CS having 88% soymeal compared to CS having 42% soymeal as protein source thus, high protein supplements have been reported to limit DMI. Deoni calves in TG were restricted their intake with 14% higher protein intake but, GE intake was 16% lesser. In contrary, DMI of HF crossbred calves in TGs were not traded off even after consuming 38% higher protein and 10% GE. This showed limitation in phenotypic plasticity of digestive system to food intake in Deoni calves in comparison to HF crossbred calves. Phenotypic plasticity of digestive system has been explained as compensating low quality diet with higher intake to fulfil nutrients requirement to the animals or not limiting the total intake even when the density of nutrients is higher. In case of Deoni calves, metabolic finity is reached either in terms of quantity or quality of the diet thus, indicated less scope to improve any productive performance of these calves.

Table.1 Chemical composition of dietary ingredients

Parameter	Green fodder Offered	Concentrate supplement	50% GNC + 50% Jaggary
Moisture (%)	80.28 ± 0.37	11.25 ± 0.23	7.05 ± 0.42
Dry Matter (%)	19.72 ± 0.37	88.75 ± 0.23	92.95 ± 0.42
Organic Matter (%)	89.61 ± 0.58	87.79 ± 0.45	93.61 ± 0.68
Crude Protein (%)	5.10 ± 0.13	15.59 ± 0.58	26.12 ± 0.23
Ether Extract (%)	1.75 ± 0.05	2.86 ± 0.36	5.35 ± 0.06
Total Ash (%)	10.39 ± 0.58	12.21 ± 0.45	6.39 ± 0.68
Acid Insoluble Ash (%)	3.09 ± 0.11	2.43 ± 0.25	0.90 ± 0.04
Total Carbohydrates (%)	82.76 ± 0.56	69.34 ± 0.66	62.15 ± 0.48
Cell Content (%)	34.27 ± 0.72	57.07 ± 0.19	84.59 ± 0.21
Neutral Detergent Fiber (%)	65.73 ± 0.72	42.46 ± 0.27	14.95 ± 0.39
Acid Detergent Fiber (%)	41.19 ± 0.99	11.03 ± 0.40	11.41 ± 0.21
Hemicelluloses (%)	24.53 ± 1.08	31.43 ± 0.29	3.54 ± 0.48
Celluloses (%)	33.70 ± 0.72	7.80 ± 0.25	8.04 ± 0.07
Acid Detergent Lignin (%)	4.99 ± 0.23	3.61 ± 0.19	2.83 ± 0.09
Non-Fibrous Carbohydrates (%)	17.03 ± 0.32	26.88 ± 0.61	47.21 ± 0.47
Gross Energy (Kcal/kg DM)	3672 ± 24	3655 ± 23	4022 ± 27

Table.2 Nutrient intake (kg/d) from total diet in different groups

Parameter	XCG	XTG	DCG	DTG	SEM	P Value
	Nutrient Intake (kg/d)					
DM	3.41^{c}	3.65^{c}	2.63^{b}	2.14^{a}	0.09	0.01
OM	3.04^{c}	3.22^{c}	2.36^{b}	1.90^{a}	0.08	0.01
CP (g/d)	3.79^{c}	5.22^{d}	2.75^{a}	3.12^{b}	0.07	0.01
EE	0.073^{b}	0.139^{d}	0.055^{a}	0.083^{c}	0.01	0.01
TA	0.362^{c}	0.436^{d}	0.277^{b}	0.241^{a}	0.01	0.01
TCHO	2.59^{c}	2.60^{c}	2.03^{b}	1.53^{a}	0.07	0.01
GE (Mcal)	12.55^{c}	13.73^{d}	9.70^{b}	8.13^{a}	0.34	0.01

Table.3 Purine derivatives excretion and Microbial Nitrogen production in different groups

Parameter	XCG	XTG	DCG	DTG	SEM	P Value
Allantoin (mM/L)	13.51[b]	18.45[c]	8.42[a]	17.70[bc]	1.42	0.01
Uric acid (mM/L)	4.22	5.44	2.44	3.67	0.97	0.23
Creatinine (mM/L)	2.00[ab]	2.87[b]	1.63[a]	3.05[b]	0.35	0.04
Total PD (mM/L)	17.74[b]	23.89[b]	10.86[a]	21.37[b]	1.92	0.01
PD index	296.29[b]	284.27[ab]	213.59[a]	210.91[a]	25.13	0.06
PD excretion (mM/d)	284.44[b]	272.90[b]	171.94[a]	169.79[a]	23.38	0.01
Duodenal flow of MBN (g/d)	239.15[b]	229.34[b]	143.09[a]	141.56[a]	20.00	0.01
Efficiency of prod. (g N/kg of DOMI)	131.31	104.94	102.84	108.05	10.35	0.24
Efficiency of prod. (g N/Mcal of ME)	35.60	26.78	28.37	27.97	2.75	0.15

Fig.1 Nutrient intake (kg/d) of nutrients in different groups

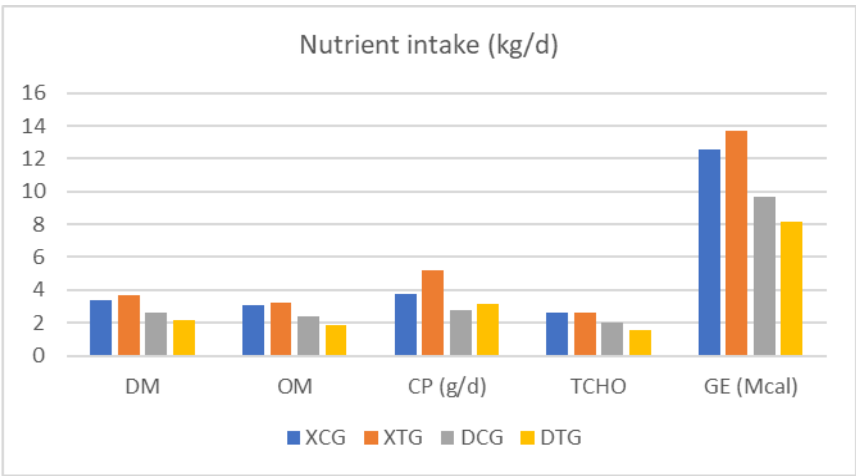
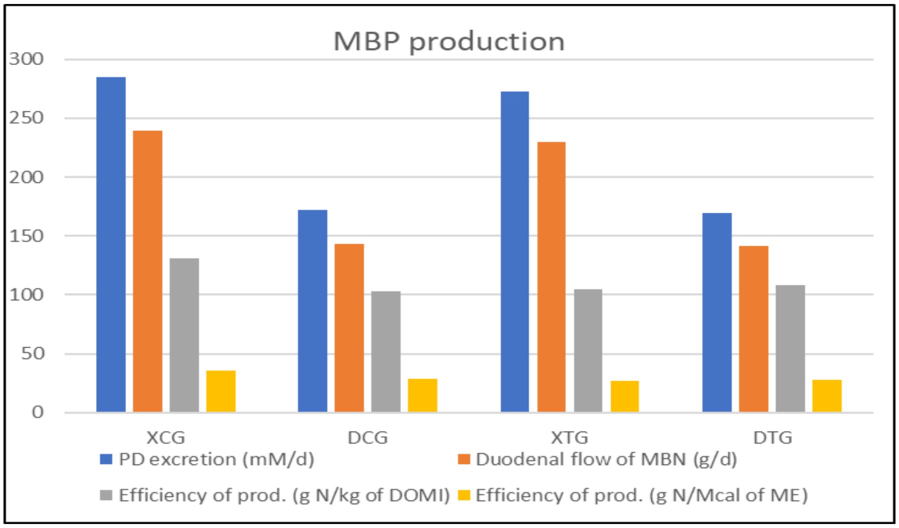


Fig.2 MBP production in different groups



It is also a classic example for less plasticity of digestive system of Deoni calves where high protein intakes are limited by the amino-peptide N in digestive system (Dewitt *et al.,* 1998).

Microbial protein production

Allantoin excretion in DTG was about 2 times higher than DCG. It was only 36% higher in XTG than XCG. This indicated higher metabolism of microbial PD at small intestine. Significantly low (P = 0.04) creatinine concentration in DCG indicated the direct relationship with body weight and less metabolically active tissue (Oser, 1965). Srinivas and Singh (2011) reported excretion of 3.0 to 3.4 mM/L creatinine. Significant (P = 0.06) breed difference was observed for the PDC index where HF crossbred calves were having higher PDC index. This indicated more MBP hydrolysed at small intestine in comparison to metabolically active tissue in the crossbred calves or vice-versa with Deoni calves. Urinary PD and duodenal flow of purine bases differs among species (Chen *et al.,* 1990) and within cattle species (Liang *et al.,* 1994). MBP production in ruminants is influenced by various biological, physical and chemical factors pertaining to animal, feed and environment in rumen (Srinivas and Krishnamoorthy, 2013). Diet composition is important in MBP yield. MBP yield on grain-based diets were higher (Singh and Srinivas, 2016) than oilseed meals (Mohanavel and Srinivas, 2016). Grains which contain more starch is a principle component of NFC and its availability influences the rumen MBP production. Quantitative availability of MBP was constant and related to size of the cow in general and, rumen in particular. MBP production in crossbred cows was reported as low as 80 g/d to 270 g/d on straw diet without or with CS, respectively (Srinivas and Gupta, 1997). MBP production efficiency was 100g/kg DOMI which was higher than the optimum suggested 30 g/kg DOMR (digestible OM in rumen) where DOMR was 65% of digestible OM (DOM) intake (ARC, 1980). Optimum MBP production for kg DOM intake would be about 50 g. In the present study, it was 2 folds more while Singh and Srinivas (2016) reported 50% efficiency on grain-based diet in Deoni cows compared to present study. Mohanavel and Srinivas (2016) reported 30 to 39% on different types of oilseed-based CS as against the present study. By and large, the MBP production efficiency observed in the post-weaned calves was high irrespective of HF crossbred or pure Deoni breed that can be attributed to diet.

Total diet intake or digestible nutrients intake in weaned Deoni calves in comparison to HF crossbred calves was hindered due to limited phenotypic plasticity of digestive system. Thus, Deoni calf response to enhanced protein and energy would be limited by its capacity to dry matter intake and protein intake.

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Compliance with ethical standards

The study was approved by the committee of ethics in Animal Experimentation.

Conflict of interest

The authors declare that they have no conflict of interests

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