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Effect of dietary incorporation of silkworm pupae meal on *in vitro* rumen fermentation and digestibility

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ABSTRACT

This experiment was conducted to study the effect of supplementation of different inclusion levels of defatted silkworm pupae meal (DSWP) on *in vitro* rumen fermentation and digestibility. Eleven concentrate mixtures were formulated with graded levels of DSWP by replacing 0 (T_0), 10 (T_1), 20 (T_2), 30 (T_3), 40 (T_4), 50 (T_5), 60 (T_6), 70, (T_7), 80 (T_8), 90 (T_9) and 100% (T_{10}) of soybean meal (SBM) protein of the control concentrate mixture. *In vitro* experiments were conducted to study the effect of supplementation of different levels of DSWP in the concentrate mixture on finger millet straw (FMS) based diets (30:70). *In vitro* total gas production (IVTGP), pH, ammonia nitrogen (NH_3-N), total volatile fatty acids (TVFA), partitioning factor (PF), microbial biomass production (MBB), metabolizable energy (ME), *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) were determined. No significant difference was observed in pH, NH_3-N, TVFA, PF, MBB, ME, IVDMD and IVOMD among treatments (T_0 to T_{10}). The results indicated that DSWP can be safely incorporated in the concentrate mixture by replacing 100% conventional protein without affecting the rumen fermentation and digestibility. Hence, it was concluded that supplementation of DSWP up to 100% had no significant effect on *in vitro* rumen fermentation and digestibility on FMS based ration.

Key words: Defatted silkworm pupae meal, *In vitro* digestibility, *In vitro* rumen fermentation, Microbial biomass, Partitioning factor

Feed cost accounts for 40–60% of the total cost of dairy production (Lawrence *et al*. 2008). The net deficit of 47% in concentrates (NIANP 2013), high cost of oil cakes and decline in availability of land under forage production have led to inadequate supply of feeds for livestock. The shortage of feed resources is one of the major constraints for animal production system in India (Samal and Pattanaik 2013). Hence, with the existing huge gap between availability and requirement of feed resources, the future hopes of feeding millions of livestock and safeguarding their food and nutritional security would depend on identification and efficient utilization of unconventional feed resources, in addition to judicious utilization of available feed resources.

Silkworm pupae, a by-product of the silk reeling industry is a rich source of proteins (50–80% CP), fats (25–35% EE), vitamins (E, B_1, B_2) and minerals (copper, iron, selenium) (Ichim *et al*. 2008). The silkworm pupae consisting of numerous biological constituents have multifaceted uses such as feed/food for animals including human beings, medicine and manure for crops. It can be a good source of protein to animals but detailed study pertaining to the optimum level of inclusion in the animal diet is scanty. Therefore, before feeding to animals, it is imperative to determine to what extent silkworm pupae can be incorporated in the animal diet. Hence, the present study was aimed to evaluate the effect of supplementation of different inclusion levels of defatted silkworm pupae meal (DSWP) on *in vitro* rumen fermentation and digestibility.

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MATERIALS AND METHODS

Sample collection and preparation: The samples of silkworm pupae meal (fatted and defatted types) were procured from Central Sericultural Research and Training Institute, Mysuru and were sun-dried for 2 days. The other feed samples (maize, soybean meal, wheat bran, rice bran, finger millet straw) used in the experiments were ground to pass through a 1 mm sieve and preserved for subsequent uses. Finger millet straw was used as a sole source of roughage in the study.

Chemical analysis: Feed samples were analyzed as per AOAC (2005) for dry matter, crude protein, ether extract,

total ash and acid insoluble ash and the fibre fractions were determined as described by Van Soest *et al*. (1991).

Experimental diets: Eleven iso-nitrogenous concentrate mixtures were prepared by incorporating graded levels of DSWP so as to replace 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% CP from SBM of the control concentrate mixture (Table 1). Subsequently eleven experimental diets were prepared by mixing these concentrate mixtures with FMS in ratio 30:70 for *in vitro* studies.

Rumen liquor and donor animal: Rumen liquor was collected from three crossbred steers (avg. body weight 496 kg), fed on a roughage (*ad lib.*) and concentrate (1.5 kg) based diet to meet nutrient (DM, CP and TDN) requirements for maintenance (ICAR 2013). Fresh and clean drinking water was offered free choice to the animals housed in well-ventilated shed with provision of individual feeding. Rumen liquor was collected from all three crossbred steers before the morning feeding and watering into a previously CO_2 flushed, pre-warmed thermos-flask and immediately brought to the laboratory. The rumen liquor was filtered through four-layers of muslin cloth and required volume was taken. Strictly anaerobic condition was maintained throughout the *in vitro* experiment.

In vitro *studies*: The *in vitro* study was conducted using eleven experimental diets which were incubated for 24 h to evaluate the effect of DSWP on *in vitro* rumen fermentation and digestibility

In vitro *gas production and substrate digestibility*: Air-equilibrated feed samples (200±10 mg) were incubated in 100 ml calibrated syringes at 39°C for 24 h with 30 ml mixed rumen suspension containing rumen buffer and rumen liquor in ratio 2:1 (Menke and Steingass 1988) with parallel incubations of blank without feed sample. The syringes were shaken at every half an hour till 2 h and thereafter, every 2 h up to 8 h to initiate proper mixing of substrate with inoculums. Each sample was incubated in triplicate over three separate runs.

Estimation of gas, total volatile fatty acids and ammonia nitrogen: After 24 h of incubation, syringes were placed immediately in ice water (4°C) to halt the further microbial activity. Net gas produced (ml/200 mg substrate) due to fermentation after 24 h was recorded after correcting corresponding blank values. Later contents of the syringes were transferred into centrifuge tubes and immediately pH was measured using pH meter. Further, contents were centrifuged at 5000 rpm for 20 min at 4°C. About 800 µl of the supernatant were mixed with 200 µl of 25% metaphosphoric acid and were stored at –20°C for further analysis of volatile fatty acids. The concentrations of volatile fatty acids were assessed (Filipek and Dvorak 2009) using a gas chromatograph (Agilent; Model 7890 A GC System) equipped with a flame ionization detector and a Agilent J&W DB- WAX GC column. Total volatile fatty acids were calculated by adding the concentration of all the individual volatile fatty acids obtained in gas chromatograph. Similarly, around 8 ml of supernatant were mixed with 40 µl of mercuric chloride and were stored at –20°C for analysis of rumen ammonia nitrogen. The concentrations of ammonia nitrogen in the supernatant were measured using UV spectrophotometer as demonstrated by Park *et al.* (2009).

In vitro *dry matter degradability and microbial protein:* In the other sets for gravimetric determination of substrate degradability, contents of syringes after incubation were refluxed for 1 h with neutral detergent solution, filtered and oven dried at 60°C for 48 h to determine the *in vitro* dry matter degradability (IVDMD). Subsequently, the residue obtained after neutral detergent solution treatment were incinerated at 600°C for 3 h to estimate the *in vitro* true organic matter degradability (IVTOMD). Further, microbial biomass production (MBB), partitioning factor (Blummel *et al*. 1997) and metabolizable energy (Menke and Steingass 1988) were calculated.

Statistical analysis: The experimental data were subjected to one way analysis of variance (ANOVA) as per Snedecor and Cochran (1994). The data were analysed using SPSS (2008) version 15.0 programme and the means were tested for the significant difference by using Ducan's multiple range test.

RESULTS AND DISCUSSION

Silkworm pupae meal is a rich source of crude protein. Fatted silkworm pupae (FSWP) meal had higher crude protein and ether extract compared to SBM (Table 2). Similar findings were reported by Tomotake *et al.* (2010), Ioselevich *et al*. (2004) and Chandrasekaraiah *et al.* (2002, 2003). The defatted silkworm pupae (DSWP) meal had higher crude protein compared to FSWP, SBM and low ether

Table 1. Ingredient composition of *in vitro* concentrate mixtures

Ingredient	Level of replacement (%)										
	0	10	20	30	40	50	60	70	80	90	100
Maize	36.0	36.6	38.8	38.0	38.0	38.0	38.0	38.0	38.5	38.5	38.0
SBM	20.0	18.0	16.0	14.0	12.0	10.0	8.0	6.0	4.0	2.0	0.0
DSWP	0.0	1.4	2.7	4.1	5.5	6.9	8.2	9.5	11.0	12.3	13.7
Wheat bran	23.0	23.0	22.0	21.0	20.0	20.0	18.8	19.5	17.0	17.0	16.5
Rice bran	18.0	18.0	17.5	19.9	21.5	22.2	24.0	24.0	26.5	27.2	28.8
Mineral mixture	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100	100	100	100	100	100	100

extract compared to FSWP (Table 2). The CP content of DSWP obtained in the present study was in agreement with the findings of Chandrasekaraiah *et al.* (2003). The higher EE of FSWP limits its utilization as protein source in ruminants (Ioselevich *et al*. 2004). Thus, higher CP and lower EE of DSWP makes it an ideal alternative protein supplement for ruminants. The CP, EE, ash, NDF and ADF content in finger millet straw were 4.98, 1.11, 10.11, 66.79 and 42.19%, respectively. The proximate principles of finger millet straw (Table 2), viz. crude protein, ether extract and total ash were in close agreement with the values reported by Chandrasekharaiah *et al*. (2011, 2012) and Sampath *et al*. (2008).

On chemical analysis, eleven concentrate mixtures prepared with graded levels of silkworm pupae meal by replacing soybean meal protein at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% had crude protein content ranging from 17.34 to 17.43% (iso-nitrogenous), thus showing not much variation in chemical composition (Table 3).

Gas production is the result of fermentation of substrate by microbes to volatile fatty acids. The mean value of *in vitro* total gas production (IVTGP) for eleven diets ranged from 180.55 to 185.55 ml/g DM (Table 4). Although higher and lower values of IVTGP were observed in T_2 and T_9, no measurable change among the treatments was observed. Higher and lower value of total gas production in T_2 and in T_9 was due to its high IVDMD (%) and low IVDMD (%), respectively. This indicated that complete replacement of soybean meal with defatted silkworm pupae meal in substrate had no adverse effect on *in vitro* total gas production. Similarly, Soleimani *et al.* (2015) also did not find any significant effect on *in vitro* gas production when gaur meal was replaced by SBM at graded levels (0, 33, 67, 100%). The mean value of *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) for 11 diets ranged from 47.39 to 48.14% and 49.23 to 49.82%, respectively (Table 4). Although IVDMD and IVOMD followed a similar trend, no significant variations were found among the treatments. This showed that incorporation of defatted silkworm pupae meal up to 100% in diet had no negative effect on the IVDMD and IVOMD. The partitioning factor for eleven diets ranged from 2.69 to 2.76 (Table 4). Partitioning factor (PF) which is the ratio of *in vitro* substrate truly digested to gas volume (Blummel *et al*. 1997) can theoretically vary from 2.75 to 4.41. The PF values obtained in this study were well within normal range reported previously for a wide range of straws (Kiran and Krishnamoorthy 2007). The MP synthesis is defined as grams of microbial CP/kg or 100 grams of OM digested in the rumen (Hoover and Stokes 1991, Stern and Hoover 1979). Microbial biomass is the major source of protein for the ruminant animals which is a source of truly available protein post-ruminally. In this study, for eleven diets, value of microbial protein production ranged from 17.87 to 19.89 mg, with no significant variation among the treatment groups (Table 4). ME values for 11 diets ranged from 8.12 to 8.25 MJ/kg DM (Table 4). Although T_2 was observed to be higher among all treatments due to higher gas volume (185.55 ml/g DM) produced by it as evident in IVTGP, no significant difference was observed among the treatments. The ME

Table 2. Chemical composition of feeds

Parameter	Defatted silkworm pupae meal	Fatted silkworm pupae meal	Soybean meal	Finger millet straw
OM	95.28	94.13	91.08	89.89
CP	68.70	60.93	47.08	4.98
EE	3.94	30.21	1.25	1.11
TA	4.72	5.87	8.91	10.11
AIA	0.52	0.30	1.80	3.35
NDF	19.56	25.40	17.70	66.79
ADF	13.00	10.89	12.50	42.19
ADL	2.75	4.18	3.49	6.69
Hemicellulose*	6.56	4.51	4.21	24.60
Cellulose	2.89	5.99	13.42	31.63
T-CHO*	22.65	2.99	42.76	83.79

DM, Dry matter; OM, Organic matter; CP, Crude protein; EE, Ether extract; TA, Total ash; AIA, Acid insoluble ash; NDF, Neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergent lignin. Hemicellulose* = NDF (%) – ADF (%). T-CHO* = 100 – (CP+EE+TA).

Table 3. Chemical composition (% DM basis) of concentrate mixtures used in *in vitro* study

Parameter	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
DM	87.66	87.26	86.83	86.46	86.08	85.69	86.31	84.94	84.538	84.13	83.76
OM	91.7	91.9	92.2	92.1	92.1	92.2	92.2	92.3	92.4	92.4	92.4
CP	17.35	17.41	17.42	17.39	17.37	17.42	17.38	17.43	17.36	17.42	17.43
EE	2.65	2.71	2.77	2.79	2.81	2.85	2.88	2.93	2.94	2.98	3.01
TA	8.29	8.13	7.8	7.88	7.86	7.79	7.76	7.68	7.63	7.56	7.58
NDF	36.14	37.01	36.25	36.88	37.60	37.40	37.99	38.09	38.76	39.26	39.35
ADF	15.16	14.35	14.60	15.02	15.30	16.03	16.20	16.38	16.68	16.60	17.34
ADL	5.09	5.08	4.92	5.34	5.54	5.61	5.92	5.93	6.25	6.37	6.67
ME* (MJ/kg DM)	11.33	11.47	11.68	11.68	11.74	11.84	11.88	12.00	12.04	12.14	12.18

DM, Dry matter; OM, Organic matter; CP, Crude protein; EE, Ether extract; TA, Total ash; NDF, Neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergent lignin; ME*, Calculated metabolizable energy.

Table 4. Effect of incorporation of graded levels of defatted silkworm pupae meal by replacing soybean meal on *in vitro* digestibility of finger millet straw based diet

Diet	Gas production (ml/g DM)	IVDMD (%)	IVOMD (%)	PF	MBB (mg)	ME (MJ/kg DM)
T_0	185.00	48.08	49.23	2.69	17.87	8.23
T_1	182.50	47.91	49.33	2.73	19.13	8.17
T_2	185.55	48.14	49.48	2.69	17.94	8.25
T_3	181. 65	47.43	48.97	2.72	18.75	8.14
T_4	181.65	47.62	49.56	2.73	19.35	8.14
T_5	182. 80	47.92	49.82	2.71	18.42	8.18
T_6	183. 90	47.96	49.71	2.74	19.57	8.21
T_7	181. 25	47.52	49.43	2.74	19.79	8.14
T_8	181. 65	47.65	49.47	2.74	19.38	8.15
T_9	180. 55	47.39	49.3	2.76	19.89	8.12
T_{10}	181.65	47.75	49.34	2.70	18.25	8.15
Mean	182.60	47.76	49.42	2.72	18.93	8.17
SEM	0.44	0.06	0.08	0.01	0.27	0.01
P value						
Linear	0.56	0.11	0.69	0.36	0.44	0.34
Quadratic	0.55	0.46	0.165	0.56	0.54	0.72

values of our study were in agreement with findings reported by Kumari *et al*. (2012) on *in vitro* incubation of roughage and concentrate in ratio of 70: 30.

The pH and total volatile fatty acids (TVFA) ranged from 6.72 to 6.79, and 44.23 to 45.21 mM/l, respectively (Table 5). Difference observed in pH and TVFA were statistically similar among treatments (T_0 to T_{10}). The pH values observed in the study were within the range (6.2–7.0) required for optimum activity of cellulolytic bacteria and fibre digestion (Orskov and Ryle 1990). Also the concentrations of TVFA were in agreement with the findings (TVFA – 46.6 mM/l) of Dung (2013) reported in high roughage based diet (80:20; roughage:concentrate) at 24 h of *in vitro* incubation. The concentration of ammonia nitrogen in eleven diets (18.59 to 19.68 mg/100 ml) was above the critical concentration (5 mg/100 ml) required for minimum microbial protein production (Table 5). Higher concentration of ammonia nitrogen was found in T_4 and differed significantly ($P<0.05$) among treatments. Although a decrease in the concentration of ammonia nitrogen was observed in treatments T_7, T_8, T_9 and T_{10}, its concentration was within the range (15–20 mg/100 ml) required for efficient nutrient utilization (Perdok and Leng 1990) and showed similar variation among the treatments.

The results of chemical composition, gas production, *in vitro* rumen fermentation and digestibility indicated that defatted silkworm pupae meal can be safely incorporated in the concentrate mixture replacing 100% of soybean meal (conventional protein supplement). However, further *in vivo* studies are required to validate the *in vitro* results.

Table 5. Effect of incorporation of graded levels of defatted silkworm pupae by replacing soybean meal on *in vitro* rumen fermentation

Diet	pH	NH_3-N (mg/100 ml)	TVFA (mM/l)
T_0	6.72	19.58^{ab}	45.05
T_1	6.72	19.50^{ab}	44.64
T_2	6.73	19.59^{ab}	45.21
T_3	6.74	19.36^{ab}	44.43
T_4	6.74	19.68^{a}	44.47
T_5	6.75	18.99^{ab}	44.82
T_6	6.76	19.21^{ab}	44.85
T_7	6.76	18.81^{bc}	44.40
T_8	6.77	18.64^{c}	44.58
T_9	6.78	18.59^{c}	44.23
T_{10}	6.79	18.65^{c}	44.62
Mean	6.75	19.14	44.66
SEM	0.02	0.07	0.41
P value			
Linear	0.683	<0.001	0.979
Quadratic	0.094	0.419	0.722

^{a,b,c}Values with different superscripts within the column differ significantly.

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