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Effect of *Bacillus subtilis* supplementation on carcass characteristics and proximate composition of satpuda poultry breed

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Abstract
The present investigation was carried out to assess the effect of feeding *Bacillus subtilis* on carcass characteristics and proximate composition of satpuda poultry breed. 120, day old, Satpuda chicks were purchased form Yashwant Aristech hatchery, Pvt. Ltd. Jalgaon, Maharashtra. They were randomly distributed into four groups T_0, T_1, T_2 and T_3 with 30 chicks in each group. The experimental chicks were reared on deep litter system in well-ventilated shed from 0-10 weeks. The *Bacillus subtilis* was added in experimental diet at the rate of 0, 0.5, 1 and 1.5 per cent for T_0, T_1, T_2 and T_3 respectively. The control group (T_0) was without *Bacillus subtilis*. The experimental ration was isoproteinous. The experiment was continued up to 10 weeks. Meat characteristics showed significant difference for dressing percentage and all the remaining parameters showed non-significant differences regarding meat bone ratio, weight of heart, liver and gizzard and also regarding cut up parts percentage. Proximate composition of breast and thigh meat showed no significant differences among all the treatments except crude protein. Crude protein for T_0, T_1, T_2, and T_3 groups was 19.81, 20.30, 21.35, 22.89 and 20.72, 20.37, 21.15 and 21.96 in breast and thigh meat respectively. It is therefore concluded that dietary inclusion of 1.5% *Bacillus subtilis* level in Satpuda poultry ration found to be better in terms of overall performance followed by 1.0% and 0.5% inclusion of *Bacillus subtilis*.

Keywords: Probiotic, *Bacillus subtilis*, carcass characteristics, proximate composition, poultry, Satpuda

1. Introduction
The intensifying income and changing consumer preferences lead to significant market opportunities for higher-value agricultural products. India is one of the largest poultry producing country in Asia. Before 1960s, from being largely a backyard endeavor, Indian poultry sector has evolved into an effervescent agribusiness spurred by domestic economic growth and consumption dynamics. The share of poultry in domestic meat production has grown swiftly. By 2006, India was producing 2.0 million metric tons of poultry-meat (Hellin and Erenstein, 2009) [5]. By 2016, India was producing approximately 3.2 million metric tons of poultry-meat (DAHD, 2016-17). And presently India was producing approximately 4.995 million tons of poultry-meat (DAHD, 2023). By 2030, it is expected to reach about 3.0 million metric tons per annum (Joshi and Kumar, 2012) [7]. The per capita consumption of meat is expected to increase in India, from its current level of 3.1 kg to 18 kg by 2050, of which 12.5 kg would be chicken (Alexandratos and Bruinsma, 2012) [1]. Poultry meat is widely accepted due to its rich taste high protein content, low fat content and comparatively economical than other meat products without disparity among regions and religions (Manning and Baines, 2004) [12]. The magnificent expansion of industry is also due to the fact that it provides the main source of animal protein through meat and eggs at cheaper rate as compared to other sources of animal protein, low maintenance cost and minimum space requirements, broilers adapt easily to almost any condition and profits are quite high. In a developing country like India, poultry plays an important role in improving nutritional status of masses, which are mostly suffering from malnutrition due to inadequate and inferior quality protein in their diet and augmenting the income of weaker sections.

While meat of chicken is having low cholesterol level; it is the best from health point of view. The whole concept of Poultry farming during the last two decades or so has undergone a sea change. The poultry industry has now emerged as a highly structured and market-oriented enterprise. Thus, the major objective of poultry farming is to increase the profit margin in poultry business by improving feed efficiency and growth rat.

1.1 Importance and Need of Study

The concept of backyard poultry farming is as old as civilization. Nearly three and half decades back, most of the chicken eggs and meat were coming from traditional poultry. Primarily the objective of free-range poultry rearing was that the birds would collect feeds available in the homestead compound or backyard. Rural poultry sector though contributing nearly 30 per cent to the national egg production, is the most neglected one. The fact is that village poultry eggs and meat fetch a much higher price than that of commercial poultry. However, 70 per cent of the eggs and meat are consumed in urban and semi-urban areas and their consumption in rural is very low (Khan, 2006) [8].

Emphasis is being given on backyard poultry keeping as a mean for nutritional security of rural people and also as a mean of supplementary income generating activity besides organic poultry production. The availability of feed to backyard poultry varies greatly depending upon season and number of birds on a specific area. Therefore, strategic supplementation of balanced feed as a part of diet over to scavenging may be beneficial for augmenting backyard meat production. The primary objective of poultry production is to reduce the feed cost as it accounts for 70-80% of total cost of production. Higher price and scarcity of conventional feed ingredients has compelled the researchers to find out the possibility of using alternate non-competitive feed ingredients in poultry feeding.

1.2 Why *Bacillus subtilis* ?

Bacillus subtilis was selected for supplementation in this study due to its superior benefits in comparison to other probiotics commonly used in poultry production. Unlike many other probiotics, *Bacillus subtilis* is a spore-forming bacterium, which gives it the unique ability to survive extreme conditions in the gastrointestinal tract, such as heat, acidity, and bile salts. This resilience ensures better colonization and stability in the gut environment, promoting a more consistent and lasting impact on the poultry's microbiome.

Its capacity to produce a wide range of digestive enzymes improves nutrient absorption more effectively than other probiotics, leading to enhanced growth performance and feed conversion efficiency. Additionally, *Bacillus subtilis* is known for its ability to produce antimicrobial substances that inhibit the growth of harmful pathogens, offering superior immune modulation compared too many non-spore-forming probiotics. This results in reduced disease incidence and lower dependency on antibiotics.

Moreover, *Bacillus subtilis* has demonstrated a stronger influence on improving carcass characteristics, including meat quality and yield, by maintaining gut health and ensuring efficient nutrient use. These combined advantages make it a better probiotic option for optimizing poultry production systems, offering greater stability, efficiency, and health benefits than other alternatives.

Objectives of Study

- To study carcass characteristics of Satpuda chickens.
- To study proximate composition of Satpuda poultry meat.

2. Materials and Methods

2.1 Carcass Characteristics

At the end of experiment five birds from each treatment were slaughtered to study meat characteristics.

2.1.1 Dressing percentage

$$\text{Dressing percentage} = \frac{\text{Carcass weight}}{\text{Final body weight}} \times 100$$

2.1.2 Weight of liver and other organs

Weight of different cut up parts of carcass *viz*. Neck, breast, thigh, drumsticks, heart, gizzard and liver was taken.

2.2 Proximate Composition of Meat

The birds were slaughtered at the end of 10th week of age. Meat proximate analysis were done by following standard procedure of AOAC (2005).

Proximate Analysis

2.2.1 Moisture Content

Moisture was determined as per AOAC (1990) method gram of sample was transferred to weighed metallic dish which was then transferred to a hot air oven at 100 ± 2 ^{0}C and dried till a constant weight was obtained. The dish was kept in desiccator for cooling. After cooling, the loss in weight was determined to calculate moisture content and expressed as%.

$$\text{Moisture (\%)} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Fresh weight (g)}} \text{ X } 100$$

2.2.2 Determination of Dry Matter (DM)

Representative samples were taken in previously weighed moisture cup trays and kept in hot air oven at 100 ± 2 °C for 24 hrs.

Dry matter was calculated as follows

$$\text{Dry matter (\%)} = \frac{b}{a} \text{ X } 100$$

Where, a = Fresh weight of sample (g)
b = Dry weight of sample (g)

2.2.3 Determination of Total Ash

Five gm of air dried samples were taken in a previously weighed silica crucibles. The crucibles along with samples were kept on heater and burnt till smoke disappears from the charred mass of samples. With the help of metal tong, the silica crucibles were kept into Muffle furnace and ignited at 600 °C for 2 hrs. Allow the muffle furnace to cool down. After 12 hrs silica crucibles containing ash were removed from the furnace and transferred into desiccator, cooled and weighed. Total ash content was expressed on DM basis and calculated as follows:

$$\text{Total ash (\%)} = \frac{a - b}{c} \text{ X } 100$$

Where,
a = Weight of silica crucible with ash (g)
b = Weight of empty silica crucible (g)
c = Weight of dry sample taken for ash (g)

2.2.4 Determination of Nitrogen and Crude Protein
Nitrogen and crude protein in samples were estimated by using Kjeldahl method. The representative samples of ration were digested in Kjeldahl flask with commercial sulphuric acid in the presence of digestion mixture ($CuSO_4$: K_2S0_4 1:9). The digested samples were then transferred in to a volumetric flask to make a suitable volume of 250 ml, cooled and out of which 25 ml sample was subjected to distillation in the semi-automatic Kjeltec distillation assembly. The ammonia released during distillation was collected into 30 ml of 4 per cent boric acid solution containing mixed indicator (0.2 per cent methyl red and 0.1 per cent bromocresol green in equal amount in 95 per cent ethyl alcohol). The ammonia collected in boric acid solution was titrated against 0.1 N HCI.

$$\text{Nitrogen (\%)} = \frac{V_1 . V_2 \times 0.0014}{b} X\ 100$$

Where,
V_1 = Volume (ml) of 0.1 N HCl used for titration of sample
V_2 = Volume (ml) of 0.1 N HCl used for titration of blank
b = Weight sample taken for digestion on DM basis
0.0014= Molecular weight of nitrogen (g) equivalent to Neutralize 1 ml of 0.1 N HCl.
Crude Protein (%) = N (%) x 6.25

2.2.5 Determination of ether extract
Fat and fat soluble components of oven dried feeds and meat samples were estimated by SOCS PLUS Pelican equipment. Solvent extraction in the extraction unit was performed in two steps. One-gram oven dried samples were immersed in the boiling solvent (Petroleum ether B.P. 40-60°C) for 20 minutes to dissolve the soluble materials. In the second step, which lasts for 30 minutes the samples were raised above the solvent surface to permit efficient washing with solvent from the condensers.
After the extraction, the condenser valves were closed by lifting the samples to the upper position. After few minutes most of the solvent got collected via the condenser in a collection vessel. At last the residue of the solvent was evaporated when the air pump was started. Ether extract was collected in previously weighed extraction cups and then weighed after cooling in desiccators. The ether extract was calculated as follows:

$$\text{Ether extract (\%)} - \frac{c - a}{b}\ X\ 100$$

Where, a = Initial weight of extraction cups (g)
b = Weight of samples on DM basis (g)
c = Weight of extraction cups with ether extract (g)

2.2.6 Determination of crude fiber
The sample after defating as mentioned above were transferred from thimbles to spout less beakers of one litter capacity and in each beaker, 200 ml of 1.25 per cent H_2S0_4 was added. It was refluxed for 30 minutes on hot plates after the boiling started and thereafter, filtered through muslin cloth. The residue was washed 5-6 times with hot water until it became acid free. The residual materials on the muslin cloth were again transferred to the respective beakers and in each beaker add 200 ml of 1.25% sodium hydroxide solution (NaOH) was added. It was refluxed for 30 minutes after the boiling started and thereafter filtered through muslin cloth and washed with hot water for 5-6 times until it became free from alkali. Thereafter, total residue was transferred in a clean dry silica crucible and dried in hot air oven at 100 ± 2 °C for 24 hr and then it was cooled in desiccator and weighed. The residue was then ignited in Muffle furnace at 600 °C for 2 hr. After 12 hr. crucibles containing ash were removed from the furnace and kept into desiccator, cooled and weighed again. Weight loss due to ignition was recorded as the weight of crude fiber.

$$\text{Crude fiber (\%)} = \frac{b}{a}\ X\ 100$$

Where, a = weight of sample on DM basis (g)
b = weight of crude fiber (g)

2.2.7 Determination of Nitrogen Free Extract
The% NFE is calculated as per given formula
% NFE = 100% - (% EE + % CP + % Ash + % CF)
Where, NFE= Nitrogen Free Extract
EE = Ether Extract
CP= Crude Protein
CF= Crude Fibre

2.3 Statistical Analysis: The data generated during the experimental period was statistically analysed by CRD given by Snedecor and Cochran (1994) [18].

3. Results

Table 1: Effect of feeding *Bacillus subtilis* on carcass characteristics of Satpuda chicken at 10 weeks of age

Parameter	Treatments				SE of Mean (±)	CD @ 5%
	T_0	T_1	T_2	T_3		
Dressing (%)	78.69	78.82	81.12	81.59	0.41	1.20
Meat Bone Ratio	14.01	14.03	14.44	14.53	0.21	NS
Heart Weight (g)	8.62	8.63	8.89	8.94	0.28	NS
Liver Weight (g)	32.34	32.39	33.33	33.53	0.24	NS
Gizzard Weight (g)	40.96	41.03	42.22	42.47	0.08	NS

Table 2: Effect of feeding *Bacillus subtilis* on cut up parts (% of dressed weight) of satpuda chicken at 10 weeks of age

Parameter	Treatments				SE of Mean (±)	CD @ 5%
	T_0	T_1	T_2	T_3		
Thigh (%)	20.48	20.51	21.11	21.23	0.30	NS
Drumstick (%)	19.40	19.43	20.00	20.11	0.41	NS
Breast (%)	32.34	32.39	33.33	33.53	0.69	NS
Back (%)	31.26	31.31	32.22	32.41	0.55	NS
Neck (%)	5.60	5.61	5.77	5.81	0.18	NS
Wings (%)	14.01	14.03	14.44	14.53	0.33	NS

Table 3: Proximate composition (%) of breast meat in different treatments

Parameter	Treatments				SE of Mean (±)	CD @ 5%
	T_0	T_1	T_2	T_3		
Moisture	71.51	71.56	71.44	71.54	0.13	NS
DM	28.49	28.44	28.56	28.46	0.13	NS
Ash	1.27	1.29	1.26	1.24	0.05	NS
Crude Protein	19.81	20.30	21.35	22.89	0.22	0.67
Crude Fat	3.23	3.42	3.46	3.46	0.17	NS
NFE	75.57	75.51	75.54	75.54	0.12	NS

Table 4: Proximate composition (%) of thigh meat in different treatments

Parameter	Treatments				SE of Mean (±)	CD @ 5%
	T_0	T_1	T_2	T_3		
Moisture	68.22	68.34	68.19	68.25	0.23	NS
DM	31.78	31.66	31.81	31.75	0.14	NS
Ash	1.36	1.39	1.34	1.35	0.06	NS
Crude Protein	20.72	20.37	21.15	21.96	0.21	0.63
Crude Fat	2.88	2.91	2.93	2.94	0.14	NS
NFE	75.75	75.67	75.76	75.76	0.20	NS

4. Discussion

4.1 Meat Characteristics

The means of dressing (%), Meat bone ratio, weight of heart (g), liver (g) and gizzard (g) for various treatment groups were recorded to be 78.69%, 14.01, 8.62, 32.34 and 40.96 in T_0, 78.82%, 14.03, 8.63, 32.39 and 41.03 in T_1, 81.12%, 14.44, 8.89, 33.33 and 42.22 in T_2, 81.59%, 14.53, 8.94, 33.53 and 42.47 in T_3 respectively (Table 1).

The statistical analysis of data revealed non-significant effect of dietary inclusion of *Bacillus subtilis* on meat bone ratio, weight of heart, liver and gizzard. However it shows significant effect on dressing percentage in that T_3 is superior over other treatments. The highest dressing (%) was observed in T_3 (81.59%) followed by T_2, T_1, and T_0. Highest meat bone ratio was recorded in T_3 (14.53) followed by T_2, T_1, and T_0. Highest weight of heart was recorded in T_3 (8.94 g) followed by T_2, T_1, and T_0. Highest weight of liver was recorded in T_3 (33.53 g) followed by T_2, T_1, and T_0. Highest weight of gizzard was recorded in T_3 (42.47 g) followed by T_2, T_1, and T_0.

4.2 Cut up parts

The per cent means of different cut up parts of carcass (Table 2) such as Thigh percentage, drumstick percentage, breast percentage, back percentage, neck percentage, wings percentage for various treatment groups were recorded to be 20.42, 19.40, 32.34, 31.26, 5.60 and 14.01% in T_0, 20.51, 19.43, 32.39, 31.31, 5.61 and 14.03% in T_1, 21.11, 20.00, 33.33, 32.22, 5.77 and 14.44% in T_2, 21.23, 20.11, 33.53, 32.41, 5.81 and 14.53% in T_3 respectively.

The statistical analysis of data revealed that non-significant effect of dietary inclusion of *Bacillus subtilis* on cut up parts percentage. High thigh percentage was observed in T_3 (21.23%) followed by T_2, T_1 and T_0. Highest drumstick percentage was observed in T_3 (20.11%) followed by 20.00, 19.43, 19.40. Highest breast percentage was observed in T_3 (33.53%) followed by T_2, T_1 and T_0. Highest back percentage was observed in T_3 (32.41%) followed by T_2, T_1 and T_0. Highest neck percentage was observed in T_3 (5.81%) followed by T_2, T_1 and T_0. Highest wing percentage was observed in T_3 (14.53%) followed by T_2, T_1 and T_0.

These findings of our present study were in accordance with those of Islam *et al*. (2004) who reported that there was no significant difference in carcass yield, weight of liver, heart, gizzard and spleen of birds fed with protexin at different graded levels in drinking water. Lactobacillus sporogenes supplementation in diet of broilers did not affect the dressing percent, weight of liver, giblet, gizzard and abdominal fat in broilers (Panda *et al*., 2005) [15]. These results were also similar to findings of Paryad and Mahmoudi (2008) [16] who revealed that there was significant increase in leg yield. Fathi *et al*. (2012) [4] reported that supplementation of Saccharomyces cerevisiae at 1.25 and 1.5g/kg in diet increased thigh percentage.

4.3 Proximate Composition of Meat

4.3.1 Proximate composition of breast meat

The per cent moisture, dry matter, ash, protein, crude fat and NFE content under each treatment were 71.51, 28.49, 1.27, 19.81, 3.23 and 75.57; 71.56, 28.44, 1.29, 20.30, 3.42 and 75.51; 71.44,28.56, 1.26, 21.35, 3.46 and 75.54; 71.54, 28.46, 1.24, 22.89, 3.46 and 75.54 in T_0, T_1, T_2 and T_3 groups, respectively. (Table 3).

Statistically the difference in proximate composition of breast meat was non-significant among treatment groups, which indicated that the *Bacillus subtilis* improved feed conversion efficiency but it does not affect the meat quality however, it shows significant results in crude protein percentage. In that T_3 is superior over all other treatments.

4.3.2 Proximate composition of thigh meat

The per cent moisture, dry matter, ash, protein, crude fat and NFE content under each treatment were 68.22, 31.78, 1.36, 20.72, 2.88 and 75.75; 68.34, 31.66, 1.39, 20.37, 2.91 and 75.67; 68.19, 31.81, 1.34, 21.15, 2.93 and 75.76; 68.25, 31.75, 1.35, 21.96, 2.94 and 75.76 in T_0, T_1, T_2 and T_3 groups, respectively. (Table 4). Statistically the difference in proximate composition of breast meat was non-significant among treatment groups, which indicated that the *Bacillus subtilis* improved feed conversion efficiency but it does not affect the meat quality however, it shows significant results in crude protein percentage. In that T_3 is superior over all other treatments. The results obtained in the present study were in agreement with the findings of Li *et al*. (2008) [11] who also reported a significant improvement in digestibility of dry matter, crude protein, calcium and phosphorus in broilers at 21 and 42 days of age when fed with probiotic alone or in combination with antibiotic. Latesh *et al*. (2013) [9] also reported increased dry matter, nitrogen, calcium and phosphorus retention in birds fed with 0.05% Saccharomyces cerevisiae, Lactobacillus acidophilus and their combination in diet. Similarly, Nawaz *et al*. (2016) [14] stated that supplementation of probiotic in diet of broilers improved crude protein digestibility. Probiotic enhances the growth of non-pathogenic, anaerobic, facultative, lactic acid producing bacterial population in the gut. Probiotics increase brush-border membrane activity, promote epithelial restitution, prevent epithelial apoptosis, protect tight junctions during inflammation, suppress electrolyte secretion during enteropathogen infection, increase expression of mucin glycoproteins, provide enzymes that may enhance host digestion of dietary nutrients (Marteau *et al*., 2004; Rioux *et al*., 2005) [13, 17].

5. Conclusion

The inclusion of 1.5% *Bacillus subtilis* level in Satpuda poultry ration found to be better in terms of overall performance followed by 1.0% and 0.5% inclusion of *Bacillus subtilis*.

Carcass characteristics of satpuda chickens: Analysis of proximate composition of carcass and carcass characteristics indicated that it shows significant results only for dressing percentage other than that all groups are statistically similar thus it can be concluded that feeding of *Bacillus subtilis* upto 1.5% doesn't have any adverse effect on meat quality.

Proximate composition of satpuda meat: The difference in proximate composition of thigh meat and breast meat among treatment groups was statistically non-significant, indicating

that the *Bacillus subtilis* increased feed conversion efficiency but had no effect on meat quality. But the curde protein content in both breast and thigh meat shows significant difference.

Conflict of Interest
Not available

Financial Support
Not available

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