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PREPARATION OF SPIRULINA AND YEAST MIXTURE FOR POULTRY FEED

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Abstract: To enhance the growth in broiler chicken, several nutritional strategies have been conducted, one of which is use of *Spirulina platensis* yeast mixture. This Mixture has high content of protein, essential amino acids, vitamins, minerals, essential fatty acids and pigments. *Spirulina platensis* is also rich in polysaccharides, which may function as prebiotic. *Spirulina platensis* increased the number of leukocytes and decreased the rate of mortality. It is capable of improving the meat quality of broilers.

***IndexTerms - Spirulina*, Yeast, Broiler Chicken, Feed, Growth enhancement, Nutrition.**

INTRODUCTION

Several nutritional methods and measures have been conducted to raise the Broiler chicken with good quality, one of which is by using *Spirulina plantesis*. This Micro algae has high content of protein, essential amino acids, vitamins, minerals, essential fatty acids and pigments. *Spirulina platensis* is also rich in polysaccharides, which may function as prebiotic (Hajati & Rezaei, 2010). *Spirulina platensis* increased the number of leukocytes and decreased the rate of mortality. Including small amount of *Spirulina* supplementation in poultry feed can make the meat quality of broiler that is increasing the contents of Eicosapentaenoic acid (EPA), Docosapentaenoic acid (DPA) and Docosahexaenoic acid (DHA) in the thigh muscles of broiler chicken (Marta, Cristina & Ali, 2017). *Spirulina platensis* has been turned out as the substitute for antibiotics to broiler diet from day of hatch to slaughter age (Rubel et al, 2019). Supplementation of this feeds in husbandry dietary improve both growth and health in poultry without any harmful side effects. The improvements like growth, feed efficiency, carcass yield, meat quality and balanced adequate diets are closely related to increase feed consumption and high nutrients utilization. Increased economic pressure exerted on the cost of production made it essential to get the greatest benefits from every unit of feed, and therefore, it became too necessary to seek new suitable sources of feeds. Broiler and breeder hens' performance shows a positive impact by the employment of yeast culture in the feed ingredient. Several researches shows that the saccharified yeast based feeds have the ability to increase the metabolization in poultry chickens and feed intake.

Mannan-oligosaccharides derived from *Saccharomyces cerevisiae* cell wall have been found to promote growth and enhance immune responses in food animals (Zhou et al, 2019). A yeast cell wall derived product, was added at adequate level to evaluate its effect on growth performance and immune response in broiler chicks challenged with lipopolysaccharide (LPS) (Wand et al, 2021 & Swaggerty et al, 2019).

Saccharomyces cerevisiae, also known as baker's yeast, is one of the most widely commercialized species and an effective adsorbent, which is rich in crude protein (40 – 45%) of high biological value, and it is also rich in vitamin-B complex(Dobrzański et al, 2005). These vitamins include Biotin, Thiamine, Niacin, etc., are extracted characterized by extracting them from the culture (Barnet Sure, 1946).

Antibiotics are chemical substances that are produced by living microorganisms, such as bacteria and green plants (Sethi, Kumar & Gupta, 2013). These are used to inhibit the growth of pathogenic microorganisms that are harmful to chickens in the digestive tract. There are microorganism are considered beneficial (Linares, Ross & Stanton, 2016) and harmful.

Researches on yeast shows that the incorporation of yeast in diet improves growth in Broilers and it is done in some poultries and animal husbandry. The dietary supplementation of yeast culture (*Saccharomyces cerevisiae*) could increase the animal nutrition, health, body weight, feed efficiency and promote the immune system in animals (Elghandour et al, 2019).

The yeast feeds are industrial byproducts which are efficient than the commercial feeds in the means of cost and the metabolizing rate. The mixture of beneficial *Spirulina platensis* and *Saccharomyces cerevisiae* enhances the growth of broiler chicken and maintains the heath condition.

Feeding *Spirulina* to Chickens enhance the Zeaxanthin which encourages the quality of meat colour, Carotenoids levels, Saturated Fatty acids. It increases the Omega-3-Fatty acids reduces the Cholesterol contents in eggs (Pestana et al, 2020).

The Use of micro algae can act as a natural color enhancers in meat and egg with Poly-Unsaturated Fatty Acids (PUFA) and improve broilers health by inducing growth of beneficial bacteria in the gut. Feeding of *Spirulina plantensis* regulates macrophage phagocytic as well as metabolic pathways leading to increased nitric oxide synthase activity. *Spirulina platensis* may enhance the functions of mononuclear phagocytic system there by increasing the disease resistance potential, Phagocytosis is a process where

a cell binds to the foreign particle and engulf it. The engulfed particle is lysed and destroyed. The process is employed by immune system cells when a foreign particle or entry of pathogens like virus or an infected cell (Batshan et al, 2001).

1. The Yeast

Yeasts are microscopic organisms belonging to the classification of fungi that can be differentiated by their relatively larger cell size, their oval, elongate, elliptical and spherical cell shapes, and the process of division. The size of yeast cells vary from one another some ranging from 5 to 8 mm in diameter. Some yeasts have been reported to grow at a low acidic pH of 1.5, in up to 18% ethanol and may grow in higher sugar concentrations (Maicas, 2020). Primary dried yeast usually refers to yeast *Saccharomyces cerevisiae*, which is grown and harvested as a nutritional yeast source. It is generally grown on sugar substrates, like molasses in aerobic bioreactors, and dried at high temperature to kill the yeast cells. It is predominantly used in the food industry for enriching it and is too expensive in the case of animal feed. Brewer's yeast is a commonly used byproduct in the brewery industry.

1.1. Nutritional Components in Yeast

Yeast is rich in several nutrients such as protein (420g/Kg in dried state), vitamins, minerals and in particularly of Vitamin B groups such as nicotinamide, vitamin B6, pantothenic acid relatively rich in phosphorous but has a low calcium content (Yoshii et al, 2019). Moreover, yeast is a rich source of important inorganic elements for animals and poultry growth. The protein of yeast is favored for feeding pigs and poultry because of their simple stomach and its value of nutrition.

1.2. The Nutritive Value of Yeast

Yeast is a rich source of protein and contains all the amino acids needed by chickens (Haiman and Frank 1994). The composition and nutritive value of dried yeast on average percentage is 95.7% for dry matter, 10.7%ash, 48.7% protein, 0.55% oil, 0.5% crude fiber, 35.5% soluble carbohydrates, 5.50% phosphorus, 2.0% potassium and 0.03% chloride, (Hamad ,1986). When compared with those of yeast and other feeds such as Cotton seed meal, Sorghum and Corn grains showed that the protein of yeast contains all the essential amino acids needed by broiler chicks.

1.3. Disease Preventive Effect of Yeast

Yeast has probiotic effect, high vitamins content, essential amino acids and high proteins quality has a preventive effect against diseases. This SCP could help in preventing the incidence of diseases at 27% or higher than the normal feed intake.

The use of antibiotics for growth promotion in poultry species has been banned in Europe and the United States (Roth et al, 2019). Replacement for the antibiotics to treat poultries without any side effects are in the study of researchers. The probiotics like *Bifidobacteria* and *Lactobacillus* are seen to have such properties in improving feed conversion efficiency (Ayanwale et al., 2006; Day, 1997), improve body weight gain and reduce mortality (Jin et al., 1997), reduce disease infection (Line et al., 1998) and stimulate the immune system (Lutful Kabir, 2009).

2.*Spirulina*

Cyanobacteria – Arthrospira platensis is the scientific name of *Spirulina* originated denoting it's helical in entire length arranged in cylindrical trichomes and are rich in proteins, vitamins, and minerals. This microalgae grows by utilizing the nutrients in the presence of sunlight (Photosynthesis). Phycocyanin, a blue photosynthetic pigment present in it facilitates this process. The mature *Spirulina* contains an appropriate percentage Protein, Carbohydrate, fat and water commonly found in the water bodies as Lakes and Ponds.

2.1. Economic Importance:

Protein and energy components of broiler feed is high and the commonly used fed such as Maize, Soybean meal too costs much thus this can be met with the cost efficient Brewers dried yeast which are industrial byproducts. By this the utilization of Brewer`s dried grain feed, the Maize and Soybean feed can be reduced and they are easily digestible with high protein content.

Chickens are mono-gastric and do not possess the enzymes needed to digest high fibre diets. Thus the diet cannot be replaced by the rice bran, wheat and brewers dried grain which are cheap high fibre ingredients for maize which costs much.

This additives paves the way to overcome the vegetable protein based high fibre that are hard to be digested by the broilers (Long staff and McNab, 1989). The ability to digest these high fibres remains the target (Oyedeji, Ajayi & Egere, 2007). A positive effect includes the cost reduction in the Broiler chick feeds at the initial growth phase.

Spirulina is an element that is produced in any type of water (clean or saline). As such, *Spirulina* is readily available in capsule or powder form. A spoonful of *Spirulina* contains essential level of protein, vitamin B1, vitamin B2, vitamin B3, copper, and iron. By its higher benefits at low cost its market is increasing day by day and farmers are seeing it as a scarcity crop. By its high nutritious property *Spirulina* is often regarded as the 'Super food'.

2.OBJECTIVE

The main objective is that the usage of yeast (*Saccharomyces cerevisiae*) in the Broiler chick's diet as the growth promoter and in health performance.

- Preparation of micro feed for broiler chicken
- Cultivation of yeast(*Saccharomyces cerevisiae*)
- Cultivation of *Spirulina*
- Preparation of cultivated yeast and *Spirulina* mixture

3. MATERIALS REQUIRED

3.1. *Spirulina*

- *Spirulina* strain
- Zarrouk's Medium (pH 9.5).

- $NaHCO_3$ 16.0
- $NaNO_3$ 2.5
- NaCl 1.0
- K_2SO_4 1.0
- K_2HPO_4 0.5
- $MgSO_4 \cdot 7H_2O$ 0.2
- $FeSO_4 \cdot 7H_2O$ 0.01
- Na-EDTA 2.5
- Trace minerals
- Conical Flasks

Apart from these you require thermometer, pH sensors, and air compressors etc. for checking various growing conditional parameters.

3.2. Yeast

YPD (Yeast Extract-Peptone-Dextrose) Medium / Potato Dextrose Broth Yeast Strain (*Saccharomyces cerevisiae*) Sugarcane Molasses.

4. CULTIVATION

4.1. *Spirulina* Cultivation Process:

The process of cultivation starts initially by filling water in the container or pond where the process is done. The water is checked to have the right pH value and alkaline by adding salts at the required rate.

4.2. REQUIREMENTS

4.2.1. Growing premise

Spirulina is grown in waterbodies. It requires partial shade sunlight (350 micro Einstein is the most suitable level for *Spirulina*) and water it may be in a pool or a container.

4.2.2. Growing facility

The growing container must be transparent and should not break easily which facilitates the exposure of the light such as aquariums.

4.2.3. Water

Water is the main constituents of the culture medium along with fertilizers. The condition of culture medium is specific for *Spirulina* thus no other organisms grow in it. Drinking water, brackish water, rainwater or water from a natural body can be used. *Spirulina* grows in an alkaline environmental condition which contains heavy metal constituents that are absorbed by it, this makes other organisms an adverse condition to grow in it.

4.2.4. Fertilizers

The culture medium is made of water and fertilizers. The medium composition may vary by the need of type of *Spirulina* to be cultivated. The composition of the medium remains same as the base of Zarouk Medium.

4.2.4.1. The following fertilizers are required

a. Sodium Bicarbonate ($NaHCO_3$) – Baking soda or Baking powder.
b. Potassium Nitrate (KNO_3) – Urea substitute (which is made up of ammonia and CO_2), below 60 mg/liter (recommended).
c. Sodium Chloride (NaCl) - Cooking salt.
d. Potassium Di hydrogenate Phosphate (KH_2PO_4) - Can be substituted with any agricultural phosphate.
e. Iron Sulfate ($FeSO_4$) - Penta hydrate, not the type used for lawns.
f. Magnesium Sulfate ($MgSO_4$) – Penta hydrate.
g. Source of calcium - lime, calcium chloride or gypsum. Soluble or crystallize type food grade fertilizer chemicals must be used. No micronutrients solution need be added.

4.2.5. Fresh *Spirulina*

The commercially available *Spirulina* live culture is bought that is a jerry cans will be easier and it is used as the starter culture. The main criteria is that the culture must be live and mature (i.e. *Spirulina* is ready for harvest) will be good.

4.2.6. pH examination

Spirulina grows in the alkaline condition (pH of 10 - 10.5) this favors its growth by showing its dominance to the other organisms (i.e. contaminators). In order to ensure the hydrogen concentration in the growth medium there are several methods the simplest way is by using the pH or Litmus paper which shows us the pH level of the culture medium by the paper's color.

4.2.7. Filtration cloth

Filtration cloth is the cloth that is used to separate the Spirulina from its growth medium, this is the process by which Spirulina is harvested. Cloth with a density of 30-40 microns in diameter, made of polypropylene, nylon or polyesters a good solution.

4.2.8. Agitation devise

Spirulina grows at the high rate where the sunlight exposure is at maximum thus it grows denser at the top part of the culture medium so the Spirulina which can't reach the top cannot grow and eventually die. In order to avoid this, agitation is done gently in clockwise or anti-clockwise.

4.2.9. Microscope
The cultural characteristics for examining the grow Spirulina can be done using microscope.

4.3. Preparing growing culture
The culture medium of Spirulina is made based on the Zarouk medium. To prepare this medium several compounds from micro quantities to large amounts such as Sulfur, Magnesium, Calcium, Boron, Manganese, Iron, Copper, Zinc etc.

4.3.1. Weigh and mix fertilizers with water
For 1 liter composition:

- **a.** 16g - ($NaHCO_3$) Sodium Bicarbonate
- **b.** 2g - (KNO_3) Potassium Nitrate
- **c.** 1g - (NaCl) Sodium Chloride
- **d.** 0.1g - (KH_2PO_4) Potassium Dihydrogenate Phosphate
- **e.** 0.1g - ($FeSO_4$) Iron Sulfate
- **f.** 0.1g - ($MgSO_4$) Magnesium Sulfate

Finally the mixture of the above i.e. the growing culture medium will be pale shade of yellow.

4.3.2. Preparing culture medium
The preparation of the growth culture is at the ratio of 1:1(for 5 litres of Spirulina 5 litres of growth culture is prepared). The prescribed amount of fertilizers must be add and fully dissolved which can be done with agitating device. In case of pre preparation the media must be stored in a container at shade and maintained at the pH level of 8 – 8.5. In some occasion the addition of the growth culture is needed where the colour of the medium is good enough to be checked for pH.

4.3.3. Spirulina farming cycles
Spirulina reproduce asexually thus by providing it an appropriate condition within 48 hrs. it double itself by reproduction. Thus after maturation the dense Spirulina is harvested and prepared for consumption.

4.3.4. Daily care of *Spirulina*

- **a.** Agitation – Must be done every 2-3 hrs. When the light source is available. The motion must be maintained at the same direction and gentle.
- **b.** Light – It is the main source of energy in algal bodies and during the starter culture exposure of sunlight must be more during which the Spirulina grows denser but direct exposure must be avoided.in rainy days artificial lighting must be provided which will be economical and efficient.
- **c.** Culture level – the main constituent of the Spirulina culture medium is water thus the Spirulina utilize it and due to presence of heat water evaporates at the minimal rate though it must be compensated. This can be measure using the ruler and maintaining the same level.
- **d.** Temperature – 30-35°C is the optimum temperature the medium should be maintained. But it can survive at temperatures up to 20-38°C during this the metabolic rate of Spirulina will drop and if sudden change in temperature it affects the organism from 'Shock' thus the temperature must be maintained at the essential rate.
- **e.** pH level – the Spirulina growth medium is maintained in alkaline pH(8 – 8.5). Certain chemical changes occurs if pH raise or lowered it favors the chances of contamination.
- **f.** Salinity – it is the amount of saltiness in the culture medium which acts as the indicator. There are several devices to check the salt level in the medium such as Refractometer. This salinity must be maintained between 15 – 20 PPT for a good growth of the organism.
- **g.** Density – This is noted by the amount of light passed through the culture medium i.e. the density increases, the amount of light passed through becomes less and the culture is ready for harvesting where the Spirulina is grown good.

4.3.5. Increasing the quantity of *Spirulina*
In the prescribed condition the Spirulina grows at higher rate and mature within 24-48 hours. If it changes the growth may vary. Thus the ratio of 1:1 is made. For increasing the growth rate at good level a simple technique is preferred. The dam like miniature setup is made where the partition within the pool is done and by noting the growth rate the partition is lifted accordingly to creating a larger space for multiplication. In case of a container the Spirulina can be moved to a larger container. By implying this method there may be a chance for pH becoming lower and by its metabolism it will reach the optimal pH. At this period Spirulina must be taken care as if in the initial stage. Examining pH level is done as it was done during the previous stage, when pH level has returned to 10-10.5, it is advisable to give it a week to stabilize itself for another yielding if needed.

4.3.6. Harvesting
This is the part where the yield is taken. Harvesting the *Spirulina* is done with a container and filtration cloth and the culture medium with *Spirulina* is poured on to it. Matured Spirulina will remain on cloth along with the medium residues, for consumption these residues must be eliminated and pH is made neutral. The Spirulina that remains in the cloth will be in the paste like texture. Before filtration process the mature *Spirulina* must be collected and can be utilized for cultivation if needed by the repetition of the same process and maintaining same ratio.

4.3.7. Consuming *Spirulina*
Consumption of freshly cultivated *Spirulina* is highly nutritious. The intake of *Spirulina* can either be mixed with food and beverages or by other means. It can be stored frozen or dried as powder for over 2 – 3 weeks approximately. (Motunrayo et al, 2020 & Soni, Sudhakar & Rana, 2017).

4.3.8. Drying *Spirulina*
Crying *Spirulina* can be stored in a clean sterile container for months and it is the most efficient way of storage. Drying process can be done by spreading the cultivated *Spirulina* over the net enabling it to dry from top and bottom by spreading it all-over in short

period of time(Preferably in a sterile area to protect it from contaminators). It takes up to 2 days to dry sufficiently. In order to speed up the process it can be dried in an oven at 40°C for 16hours or at 60°C for 4 hours (lower the temperature of drying, higher the nutritional value).

4.3.9. Yeast cultivation

The freshly cultured yeast is dehydrated and dried along with its fermentation ability. This dried yeast is made into a small spheres and dried continuously till the water content becomes 8% but the capacity of its fermentation is maintained. The materials involved in making of the dried yeast include the molasses and some nitrogen-containing chemicals. After sterilization it is grown at a constant temperature and making it yeast sludge then granulated in drying bed at low temperature. (Hahn-Hägerdal et al, 2005).

5. MOLASSES PREPARATION

This is the main concept in fermentation where the yeast utilize the sugar source in the substrate to ferment. For this process the pretreating of the substrate is needed as the yeast cannot utilize the starch as it does not contain any catalysts to hydrolyze it to sugars. Other than this there are certain nutrients, salts and minerals for growth which are added to the substrate before sterilizing it and the other nitrogen sources such as Phosphoric acid for Phosphate and Nitrogen for Ammonia is added then. These supplements are independently fed for fermentation while the molasses is in the separate tank to sustain sugar fermentation for better pH control. The culture yeast seeds are kept in a sterile space in order to avoid contamination from other wild type yeasts present around in a sterile jars to grow and by performing several steps it is moved from these jars to tanks for fermentation. Finally the stock yeasts are separated from the fermented products and stored in refrigerated tanks for further cultivations. (El-Gendy, Madian, & Amr, 2013).

6. CULTURE PREPARATION

The developed seeds in the jars are moved to the bigger fermenters then it is followed by molasses and Ammonia, Phosphoric acid, Minerals at controlled rate. This rate enables to boost yeast cell multiplication but this process is not completely sterile. Though the pressurized tanks are not used in means of sterility, steaming of pipes, tanks and equipment are done to ensure aseptic conditions.

7. FERMENTATION

Initially this process starts with the addition of seed yeast liquid and some quantity of water which comes around 33% of the fermentor capacity further addition of the molasses and other nourishing supplements make up its last volume. And during the processes of fermentation a quantity of supplements are added to enhance the rate of yeast cell population. During fermentation process the cell population increases around 5 – 8 times. Aeration is provided during fermentation by pumping the fermentation liquid from bottom or using inner cooling curls. Thus the regulation of supplements, Airflow, pH(4.5 – 5.5) and temperature(30°C) are keenly monitored and controlled throughout the process.

8. YEAST EXTRACT PROCESS

Final process is the separation of the stock from the fermenters where the rotators are washed in water and in this process of squeezing the yeasts get heated up so it must be maintained in a cool temperature (around 7°C) with ventilation in tank till the packing process.

Table 1: Composition of *Spirulina* Yeast feed

S.No.	*Spirulina* composition (in Percentage)	Yeast composition (in Percentage)	Total composition (in Percentage)
1	40	60	100
2	20	80	100
3	30	70	100
4	10	90	100

RESULT:

The culture is the filtered and dried with hot air or in the sunlight. Microalgae have been shown to be more efficiently concentrated when harvested in the exponential phase compared to the stationary phase where the *Spirulina* floats as the lipid content would be higher and lighter than the cultural medium. Then the yeast cells are recovered from the fermentor and separated using the centrifuge. It is again concentrated with the filter press. The filtered cake is blend by adding some emulsifiers, water and oils and dried. After drying the final product is packed in vacuum. The table shows the composition of the yeast and Spirulina for the chicken feed **(Table 1)**.

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