A study on the effect of environmental temperature with relation to feed intake, mineral mobilization and respective incorporation into the egg yolk of Lohmann Brown egg layer chickens given purified lignin at the Neudamm farm in Namibia.

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Abstract

It is of knowledge that the usage of antibiotics leads to development of antibiotic-resistance by pathogenic bacteria which pose a major threat to both animal and human health. Bio-stimulators can replace the use of antibiotics as growth promoters and exert positive influence on metabolic processes. Between June and July, 2013 a study was conducted on the effect of purified lignin on egg layer chickens. The objective of this study was to assess the effect of purified lignin on Lohmann Brown egg layer chickens under Namibian environmental conditions. The feed additive made of purified lignin, containing humic acid, pH 8.5-10, and 14% humidity which is soluble in water was used in this experiment. Lignin at concentrations of 60mg kg-1, 50mg kg-1 and 40mg kg-1 diluted in water were used to feed 1446 chickens (482 each experimental group), while 489 served as the control group), for 17 days with 10 days interval at 18°C and 23% humidity. Feed intake was evaluated; eggs from both groups were harvested, sorted and graded in different categories daily. Egg content from both groups and the presence of microorganisms in the gastro-intestinal gut were assessed. Results revealed that experimental chickens had low feed intake (4.3kgs less) and produced more eggs graded as extra-jumbo and jumbo, with less egg fats and oil content (g/100g) - 17.4

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for experimental group; 19.4 control group). No gastro-intestinal microbial organisms were found. Purified Lignin is suggested to improve digestibility and feed conversion. The use of 50mg kg^{-1} led to reduced feed intake in the experimental group despite low temperature while increasing the mobilisation of Fe, (mg/kg) - 17.7; 13.7; Se mg/kg - 0.68 and 0.89; protein into egg yolk (g/100g) - 19.1; 17.1. Evidently, lignin can be used in the poultry industry as a feed additive and bio-stimulator to increase Iron (Fe) uptake which in return increases the level of haemoglobin and Selenium (Se) which serves as an antioxidant, while concurrently reducing microbial meat contamination and replace the use of antibiotics as feed additive.

Keywords: Bio-stimulators, feed intake, microbial organisms, meat contamination, environmental temperature

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1 Introduction

There is a wide tendency among animal scientists to resort to the use of bio-stimulators towards substituting the use of chemicals and pesticides to improving agricultural yields. It happens in response to the announced banning of the use of synthetic growth promoters in farm animals due their residual effect on consumers and resistance build up by pathogens or bacteria (Miles, et al, 2006). This measure has a blessing through the European Union (EU) regulations imposing restriction on the use and production of pesticides, for example; in order to drive the demand to searching for new and effective substances which are environment friendly.

In attempt to reduce the cost of animal and crop production several studies carried out have been focusing on the effect of biologically active ingredients including bio-stimulators. Active ingredients of bio-stimulators comprise many organic compounds (phenols, vitamins, polysaccharides, betaines), growth regulators, algae, humus, extract from grapefruit, garlic and also macro and micro elements (Gawrońska et al. 2008, Przybysz 2010, Truba et al. 2012. Other components like salts of trace elements, tissue preparations, ferments, amino acids, synthetic and natural hormones, also have been applied as bio-stimulators in the field of animal production (Gladkov et al., 2011), proved to be safe both for human beings and for the environment.

These macromolecules have capacity to trigger direct influence upon metabolic processes in the organism of poultry and speeding-up growth thus increasing meat production; improving egg quality through mobilization of macro and micro elements in egg yolk; improving feed conversion and natural resistance to diseases and stress caused by both internal and external factors (Gladkov et al., 2011). Significant number of growth promoting and herbal products is progressively used in poultry industry to cause lower feed intake, trigger higher growth rate, higher live weight gain while producing diseases resistance (Niwas and Singh, 2014).

The use of bio-stimulators as an option to replace chemicals seems to be effective in different fields of agriculture, although some of the mechanisms involved in the process have got no scientific explanation so far (Gawrońska and Przybysz, 2011). Moreover, it is known that bio-stimulators affect a number of physiological and biochemical changes in plant cell metabolism, through photosynthetic activities by increasing assimilation area, resulting on higher rate of photosynthesis and higher chlorophyll content (Gawrońska et al. 2008 and Przybysz et. al. 2008).

Other evidences of positive effect of bio-stimulators in crop science was revealed by Wrochna et al. (2008) who indicated that bio-stimulators increased activity of antioxidant system during salt stress while Cambri et al. (2008) found out that certain changes in some biochemical and physiological processes can affect gene expression.

2 Problem Statement

Usage of antibiotic as growth promoters is under intense scientific and public scrutiny because their use has been linked to the development of antibiotic-resistant pathogenic bacteria, which pose a threat to human health (Smith et al., 2003). Different strains of antibiotic-resistant like E. coli have been isolated from the poultry and poultry meat products in several countries (Said et al, 2013).

Food safety is threatened by pathogenic bacteria causing foodborne diseases such as Campylobacter, Salmonella, and E. coli (Rybolt, et al). The critical point of bacterial contamination of poultry products occurs at the slaughterhouse, when pathogens in the intestinal contents make contact with chicken carcasses (Heyndrickx et al., 2002). The meat contamination especially in chickens during the slaughter at abattoirs needs to be addressed through the use of bio-stimulators in replacement of antibiotics as certain microorganisms resist to its effect.

Experiments on Purified Lignin were conducted in Russia, Canada, and Europe under certain climatic conditions. Side effects and complications when using P. Lignin as per instructions are not observed therefore, the effect of Purified Lignin in egg layer chickens has never been studied in Namibia. The Namibian Ministry of Agriculture, Water and Forestry has certified and authorised for use of Lignohumate KD after it has been analysed by the Analytical Laboratory Services, Northern Industrial, Windhoek, Namibia (Tel. 061 21 0132, e-mail: analab@mweb.com.na).

2.1 Characteristics of Lignohumate®

Purified lignin is presented in form of powder; 100% soluble in water and therefore, not causing nozzle clogging. It can be used in drip feed systems. It constitutes a raw material âĂŞ containing lignosulfonate (product of wood processing) with high content of sulphur in organic bond form (more than 3%) and it has high concentration of active substance (90%). Besides humic acids (80-85%), the preparation also contains fulvic acids (15-20%) which is known to be biologically active (there is a more vivid stimulating effect both in plants and microflora in general as compared with humic acids). Thanks to a more active compound of fulvic acids and microelements, the absorption of protein is accelerated.

2.2 Different Lignin by-products

Indulin, a Purified Lignin by-product from the paper industry (Ross et al., 1986), has been shown to improve weight gain and feed efficiency in broilers (Ricke et al., 1982). Canadian scientists developed Alcell Lignin (through Alcell Technology Inc., Montreal, Canada) containing phytogenic material that inhibited the growth of aerobic bacteria in the cecum of rats and inhibited the in vitro growth of *E. coli Pseudomonas* and *Staphylococcus aureus* (Nelson et al., 1994).

Russian scientists developed the technology for the production of high-concentration feed additives made of Purified Lignin, containing phytogenic material under the trade name Lignohumate KD (Gladkow, 2011). Purified Lignin is produced in dry form, as a dark brown powder with a slight smell of vanillin, a pH 8.5-10, and a moisture content not exceeding 14%, soluble in water in any proportion.

The liquid form is also dark brown, 20% solution, containing up to 90% of salts and low-molecular humic acids, macro-elements, and a number of microelements combined with the humic acid molecule. Lignohumate KD provides a bio-stimulating effect on metabolic processes in animal organisms, increases enzyme activity, synthesizes proteins and carbohydrates, stimulates the pancreas and the production of immune competent cells and improves the digestibility of feed components as well (Gladkov et al., 2011).

2.3 Study objectives

Different amounts of P. Lignin have been used in different climatic conditions and all reported results are directly related to specific instructions in accordance with environmental conditions (cold weather). The main objective of this study is to investigate the adequate amounts of purified lignin to serve as probiotics to be recommended for use under Namibian environmental temperature conditions to improve egg production with minimal feed consumption.

2.4 Mechanism of action

Biological activity of humus substances is related by scientific researchers to affect the oxidation and reduction processes due to the presence of chemical groups in their compositions, which play the role of hydrogen carriers and oxygen activators, thus stabilizing intracellular respiration in living organism (info@humate.spb.ru, www.humate.spb.ru)

3 Methodology

Different proportions of purified lignin (Lignohumate KD) were tested in Lohmann Brown breed (egg layer chickens): 40 mg/kg, 50 mg/kg and 60 mg/kg of chicken live weight. A total of 482 chickens were supplied with Lignin for 7 days in 10-day intervals, which was allocated via a 3 m high reservoir filled with solution that was released to chickens through gravity. The temperature and relative humidity were measured on a daily basis-early morning (08:00) and peak of the day (14:00). The facility that kept the chickens was divided into two blocks of cages placed in batteries, totalling 971 heads (482 experimental group/489 experimental groups). Each cage contained 5 chickens-one chicken less than cage-carrying capacity. The chicken coop was located along the road to staff houses and was well-ventilated and supplied with tap water. Both the control group and the experimental group were subjected to stress for 10 minutes on a daily basis for 5 days in order to evaluate P. Lignin's ability to trigger resistance to stressful situations. Feed intake was calculated on a daily basis.

Eggs from both groups were collected daily at 14:00h and sent to an egg-sorting unit. Eggs were categorised based on their weight using a sorting machine as Jumbo, X-large, Large, Medium, or Small. Egg samples from both the experimental and control groups were harvested at the end of each trial period (17-18 days) and sent to the laboratory for macro and microelements analysis, and protein and fat analysis.

Samples of chickens from the experimental group were taken to the Central Veterinary Laboratory where, the presence of microorganisms in gastro-intestinal contents was evaluated. Blood samples were also taken to the Central Veterinary Laboratory for pathogenic agents screening. Chicken manure was used for teaching how to prepare compost as organic fertilizer.

3.1 Limitations and weaknesses of the study

Given the conditions of the studies, the nocturnal peak of temperatures were not evaluated a fact that may limit the observation and respective registration. The location of the coop favored frequent visits by staff members, students. Noise produced by operating machinery such as cars, an electric generator, wind, etc. affected the experiment to such extent that it was not possible to accurately measure the stress. Given the quality of the study it would have been expected that highly sensitive scales be used and the length of daylight also be recorded, which was not the case with this study.

Chickens frequently died which reduced their overall number resulting in recalculations. Taking into account that the study was conducted by lecturers and one student who have a very limited time, due attention to all phases of the experiment was needed but unfortunately it was not fully paid to. It is also important to indicate that variables like minerals and protein contents were not analysed when 60 g/kg were used (limited internal capacity).

4 Results and Discussion

Based on the outcomes of our studies whilst taking into consideration the pointed out weaknesses of the experiments, further studies with accurate observations are still required as there is need for adjusting certain parameters like the temperature; feed intakes evaluation in different feeding periods (mornings, afternoons, evenings and nights).

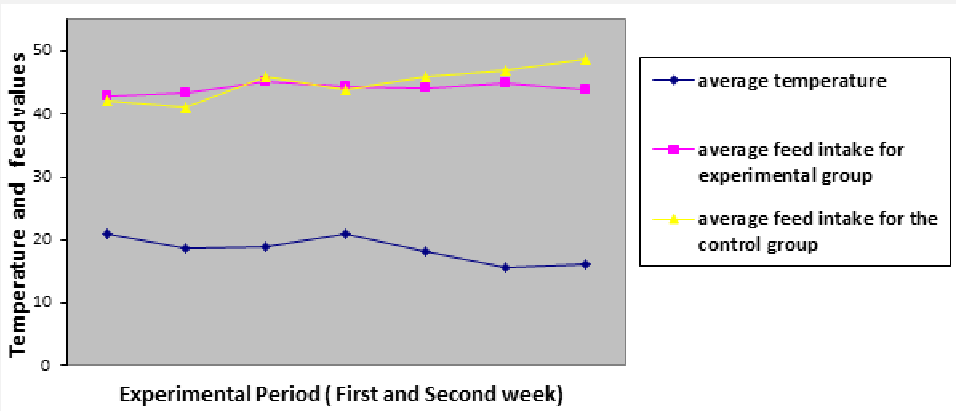

Figure 1: Effect of 50mg purified lignin (Lignohmate) per kg of egg layer chicken under environmental temperature for the first and second week of the experiment.

The feed consumption as a function of temperature shows that chickens fed with purified lignin diluted in water is less compared to non-lignin fed chickens (control group), although they seem to share similar behaviors related to the effect of environmental conditions. These findings seem to have direct dependence on the variation of temperature; leading to relatively high feed intake when temperatures are low and vice-versa. These observations can suggest that lignin triggers the occurrence of biological activities thus improving digestibility rate (Figure 1).

Table 1 screens out the results of studies carried out during the third and fourth weeks taking into account temperature as a function of egg quality.

Table 1: The effect of 40 and 50mg/kg of purified lignin on mineral mobilization and incorporation into Lohman Brown egg yolks

Minerals	Purified Lignin (40mg/kg)		Purified Lignin (50mg/kg)	
	Experimental Group %	Control Group %	Experimental Group %	Control Group %
Potassium (K)	0.13	0.12	0.23	0.22
Magnesium (Mg)	0.01	0.01	0.02	0.02
Calcium (Ca)	0.05	0.05	0.02	0.01
Phosphorus (P)	0.21	0.21	0.20	0.19
Manganese (Mn)	0.23	0.26	1.047	1.05
Iron (Fe)	18.8	16.8	17.7	13.7
Copper (cu)	0.61	0.62	1.00	0.88
Zinc (Zn)	11.2	11.6	13.7	12.88
Chromium (Cr)	0.07	0.07	0.19	0.15
Selenium (se)	0.70	0.56	0.89	0.69
Cobalt (Co)	0.01	0.01	0.02	0.02
Sodium (Na)	0.14	0.13	0.08	0.07
Protein g/100g	12.7	12.4	19.0	17.1
Fat & Oils g/100g	0.00	0.00	17.0	19.4

The results suggest that the optimal amount of lignin as a feed additive which produces observable effect is situated in the range between 40mg/kg to 50mg/kg live weight (Figure 2 and Table 1). Based on the few published studies, animal responses to purified lignin seem to depend on dosage, animal species, type and source of the lignin product. More research is required before establishing conclusive benefits of purified lignin on animal performance and health (Baurhoo et al., 2008).

It is important to make reference that at the beginning of the experiment with the use of 60mg of lignin/head, 6 (six) chickens died 4 (four) from the control group and 2 (two) from the experimental group. A blood sample analysis by the Central Veterinary Laboratory revealed the presence of one of the Virus strains causing Newcastle Disease affecting poultry unit. The experiment did not make remarkable conclusion about the effect of purified lignin as an anti-stress agent as near the coop there is a road sourcing constant noise from pedestrians and passing vehicles, which have interfered with the studies.

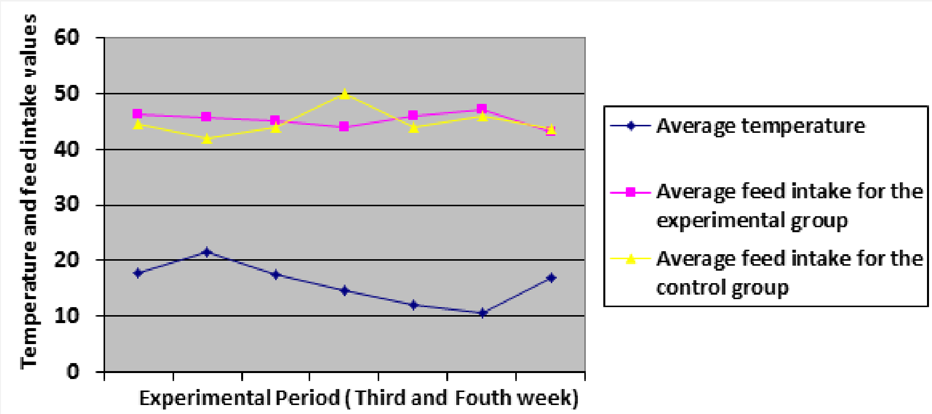

Figure 2: Effect of 60mg purified lignin (Lignohmate) per kg of egg layer chicken diluted in water and environmental temperature for the third and fourth week of the experiment.

Further, studies were aimed at screening out the effect of purified lignin as feed additive on macro and micro elements mobilization into the egg yolk, when used in different proportions (40mg/kg and 50mg/kg respectively), see the table below.

The probiotic effect of added lignin in feed helps chickens to make use of available nutrients thereby consuming less feed despite the effect of lower temperature conditions - increased feed conversion rate (Table1). The current data are in agreement with those findings in few published studies, animal responses to purified lignin seem to be dependent on dosage, animal species and type and source of the lignin product as indicated by Baurhoo et al. (2008).

According to the results on the table, the administration of 40 mg/kg and 50 mg/kg does not seem to produce any significant effect on mineral mobilization into egg yolk content. Perhaps the more purified lignin is supplied to chicken, the more iron is mobilized into the egg yolk. In summary, the variation of lignin supplementation between the 40 mg/kg and 50 mg/kg does not seem to cause significant effect in mineral concentration within the egg yolk content. The administration of 50 mg/kg of lignin can be advisable for feeding egg layer chickens whose eggs will be supplied to people suffering from anemia as it leads to an increase in iron concentration in the egg yolk. No microorganisms were found in the gastrointestinal gut content in the experimental group of chickens, which is completely abnormal. This fact may be attributed to the effect of P. Lignin, which killed all microorganisms, (Phillip et al, 2000), a fact implying that Purified Lignin has the potential to eliminate microorganisms from the gastrointestinal gut (Nelson et al., 1994).

Purified Lignin can probably be recommended for use in the poultry industry for the elimination of microorganisms in poultry products (prior to slaughter) in order to avoid bacterial contact with chicken carcasses. Furthermore, the increase of purified lignin supply can lead to an increase of protein levels in the egg yolk or improve the growth rate, a fact supported by Glakov et al. (2011). It is suggestible that P. Lignin be considered as a growth factor, and replaces the use of BSTT (as a growth factor) in beef and poultry industries.

Given that this study was conducted for the first time in Namibia and probably in the African continent, additional and more accurate studies that would include a number of variables are recommended in order to clarify a broad range of effects of P. Lignin and its applications under the African environmental conditions.

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