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## Editorial Team

# Volume 12 (1); January 25, 2022

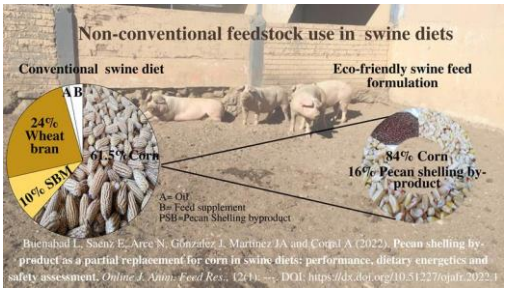
**Research Paper**

## Pecan shelling by-product as a partial replacement for corn in swine diets: performance, dietary energetics and safety assessment

Buenabad L, Saenz E, Arce N, Gonzalez J, Martinez JA and Corral A.

*Online J. Anim. Feed Res.,* 12(1): 01-06, 2022; pii: S222877012200001-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.1

**Abstract**




The objective of the present study was to evaluate the pecan shelling by-product (PSB) grindings as partial replacement for corn in swine diets and its exerting effect on sows' performance. Seventeen pregnant sows (210 kg of initial body weight) were used in a 14-d trial to evaluate the effect of partial replacement of corn with PSB on performance, dietary energetics, and safety. Treatments consisted of corn-soybean meal-based diet containing 0 or 10% of PSB in the diet, where the later replaced 0 or 16% of corn (as-fed basis). Ether extract (EE) content on PSB averaged 185 g/kg. Treatments including PSB had 1.9 times the EE compared to the treatment with only corn-soybean meal. Replacement of 16% of corn with PSB increased the estimated digestible (DE) and metabolizable energy (ME) values of the diet by 218 and 230 kcal/kg compared to control diet (90% DM basis), respectively. The higher predicted DE or ME intake for sow fed on PSB diet were 500 or 529 kcal/day compared to control diet, respectively; however, no differences on average daily gain (0.52 kg/d) between dietary treatments were observed. Neither was feed refusal for any dietary treatment nor difference on finishing their respective feed allowance. Sows consumed pecan shell at level of 0.75 g/kg of body weight daily, approximately. Intestinal activity was not affected by PSB consumption and fecal score averaged 3.0. Partial replacement of corn with PSB increased the estimated DE and ME for gestating diets by 7%, without influencing performance and intestinal function. The pecan shelling by-product supplementation at 10% inclusion level into a corn-soybean meal-based diet does not affect performance and its addition to the diet contributes to a more sustainable use of locally available feedstock in swine feeding.
**Keywords**: By-product, Corn-soybean meal, Pecan; Pigs, Sows diet.

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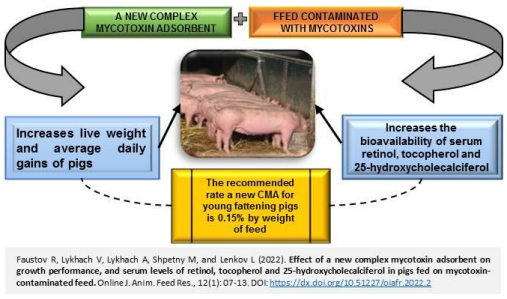
**Research Paper**

## Effect of a new complex mycotoxin adsorbent on growth performance, and serum levels of retinol, tocopherol and 25-hydroxycholecalciferol in pigs fed on mycotoxin-contaminated feed

Faustov R, Lykhach V, Lykhach A, Shpetny M, and Lenkov L.

*Online J. Anim. Feed Res.,* 12(1): 07-13, 2022; pii: S222877012200002-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.2

**Abstract**




Faustov R, Lykhach V, Lykhach A, Shpetny M, and Lenkov L (2022). **Effect of a new complex mycotoxin adsorbent on growth performance, and serum levels of retinol, tocopherol and 25-hydroxycholecalciferol in pigs fed on mycotoxin-contaminated feed.** Online J. Anim. Feed Res., 12(1): 07-13. DOI: https://dx.doi.org/10.51227/ojafr.2022.2

The objective of the present study was to investigate the effects of commercial analogue mycotoxin adsorbent (CAMA) and Gepasorbex as a new complex mycotoxin-adsorbent additive on growth performance and serum retinol, tocopherol and 25-hydroxycholecalciferol concentrations of fattening young pigs (n=90), which fed on the combined feed contaminated with mycotoxins. Animals were randomly allocated to 3 groups with 30 heads in each: the first group of pigs (control) fed the basic diet in grower and finisher periods; the second group fed the basic diet with the 0.15% by weight of feed of the mycotoxins-adsorbent as commercial analogue; the third group fed the basic diet with the addition of 0.15% by weight of feed of Gepasorbex, a new compound of mycotoxin-adsorbent additive. On the 48$^{th}$ and 88$^{th}$ days of fattening, blood samples were taken from the pigs for testing the vitamins A, E and 25-hydroxycholecalciferol levels. Piglets from third experimental group followed by group 2 animals showed better growth rates and significantly ($P<0.001$, $P<0.01$, respectively) exceeded analogues from the control group in live weight and average daily gain in all age periods which showed a noticeable positive effect of diet's mycotoxin adsorbents, especially Gepasorbex, on animal growth rates. The results of the laboratory study showed that in the pigs from third experimental group, which fed on Gepasorbex with compound feed, the serum concentrations of retinol, tocopherol and 25-hydroxycholecalciferol were within the biological reference interval and significantly exceeded ($P<0.05$)

similar indicators from the control group in both periods. The results of the experiment showed that when animals are subjected to diets contaminated by mycotoxins, use of a new complex action preparation like Gepasorbex can mitigate the negative impacts of mycotoxins on animal performance, and is more capable to remove mycotoxins without binding to dietary vitamins.
**Keywords**: Gepasorbex, Mycotoxins, Retinol, Tocopherol, 25-hydroxycholecalciferol.

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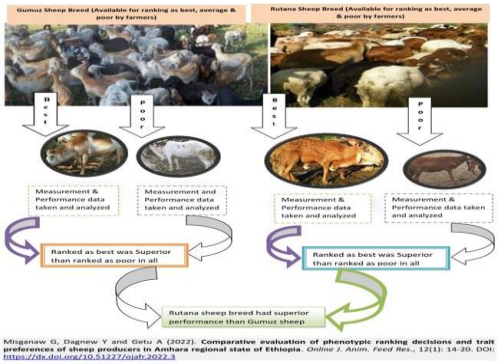
**Research Paper**

## Comparative evaluation of phenotypic ranking decisions and trait preferences of sheep producers in Amhara regional state of Ethiopia

Misganaw G, Dagnew Y and Getu A.

*Online J. Anim. Feed Res.,* 12(1): 14-20, 2022; pii: S222877012200003-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.3




**Abstract**
This study was aimed to investigate comparative trait preferences of farmers' and selection practices for Rutana and Gumuz sheep breeds in existing production system. Ranking method, such as direct and own-flock ranking experiment with sheep keepers were carried in smallholder and large-scale production systems. Appearance, coat colour and fast growth rate were important traits in selecting breeding rams in smallholder system where as fast growth rate, tail length and appearance were preferred in large-scale system. Mothering ability, multiple births and lambing interval were most preferred for ranking breeding ewes in both systems. The mean of all objectively measured body conformation traits and body weight varies significantly across the farmers ranking categories and in line with their selection decision. In Gumuz sheep breed the average body weight of ewes which ranked as best was superior to rank as poor (34.5 kg vs. 26.7kg); whereas, in Rutana breed it was 39.4 kg vs. 29.5 kg (P<0.05). The farmers' breeding objectives were improving reproduction, conformation and growth traits, which can increase net cash income per flock through increased number of marketable animals for meat production. Therefore, considering meat production traits is recommended as feasible strategy for future for Gumuz and Rutana sheep genetic improvement and conservation program.
**Keywords**: Breed improvement; Conservation; Gumuz sheep; Ranking experiment; Rutana sheep.

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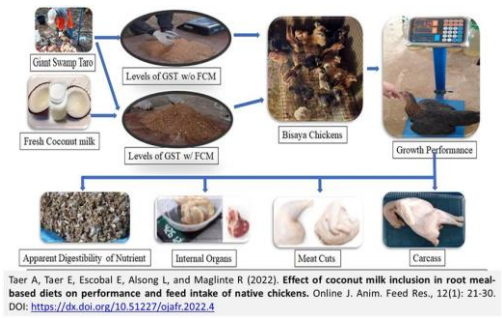
**Research Paper**

## Effect of coconut milk inclusion in root meal-based diets on performance and feed intake of native chickens

Taer A, Taer E, Escobal E, Alsong L, and Maglinte R.

*Online J. Anim. Feed Res.,* 12(1): 21-30, 2022; pii: S222877012200004-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.4




Abstract
In pursuit of feed cost reduction for chickens, a reduced feed intake strategy was proposed, but how this strategy works without compromising the chicken performance rarely explored. This study proposes the satiating effects of fresh coconut milk (FCM) as dietary fat for giant swamp taro meal-based (GST) diets on the performance and feed intake reduction of native chickens. One hundred eighty Bisaya native chickens have been grouped into FCM-free and FCM supplemented groups further divided into 0%, 25%, and 50% GST sub-groups of ten chickens in each sub-group. The experiment was laid out in a 2 × 3 factorial in a completely randomized design. The feeding trial started on week 5 post-hatch and terminated on week 12 post-hatch. The average daily feed intake (ADFI) and weight gain (WG) of chicken at weekly intervals were highly significant among FCM as well as GST treatments during weeks 5–7, also the final ADFI and WG. However, no remarkable differences of ADFI and WG in weeks 8–12 feeding. The bodyweight of chickens was highly significant among the FCM group and levels of GST were consistently observed throughout the study period. Neither the FCM nor GST groups differ the values for FCR. However, a bit higher FCR for FCM supplemented and 0% GST treatments. Slaughter weight, meat cuts (breast and thigh) and organ weight (liver, gizzard, and heart) was uninfluenced by either FCM or levels of GST. FCM supplemented diet had higher apparent digestibility of crude ash than FCM-free. 25% GST meal inclusion had higher apparent digestibility of crude fiber compared to 0% and 50% GST. No remarkable FCM×GST interactions in all parameters tested throughout the trial. In conclusion, supplementation of fresh coconut milk to giant

swamp taro meal as replacement to maize did not reduce feed intake of chickens but rather increased body weight, weight gain with marginal improvement in FCR. The 50% giant swamp taro replacement to maize compromises overall performance of native chickens. However, supplementing fresh coconut milk to giant swamp taro meal can replace maize up to 25% that gives a better body weight, weight gain, and feed conversion ratio.
Keywords: Bisaya native chicken, Coconut, Dietary fats, Feed efficiency, Root crops.

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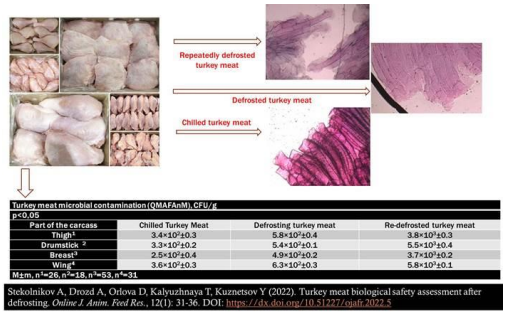
**Research Paper**

## Turkey meat hygiene and biological safety assessment after defrosting

Stekolnikov A, Drozd A, Orlova D, Kalyuzhnaya T, Kuznetsov Y.

*Online J. Anim. Feed Res.,* 12(1): 31-36, 2022; pii: S222877012200005-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.5

Abstract
Violations of temperature and humidity storage conditions and the intensive development of lactic acid putrefactive microorganisms (micrococcus, yeast, and mold fungi) lead to destructive changes in muscle tissue. The studies established the effect of single and repeated defrosting of turkey meat on the increase in the number of microorganisms that cause spoilage of products and being criteria for meat hygiene and biological safety. As a result of the study, there were no detecting bacteria of the genus *Salmonella* in a sample of 25g of chilled, defrosted, and re-defrosted turkey meat. Also, there was no detecting growth of *Listeria monocytogenes* and bacteria of the *Escherichia coli* group in a sample of 1g of the product. On the other hand, significant changes were in the dynamics of microbial contamination in terms of the number of mesophilic aerobic and facultative anaerobic microorganisms. Thus, in cooled samples, the total microbial contamination was 3.2×102±0.2 colonies of forming units per 1g of product. In samples of defrosted turkey meat, this indicator increased 1.8 times and amounted to 5.6×102±0.4. However, the obtained value did not exceed the maximum permissible, regulated in the normative and technical documentation. Samples of re-defrosted turkey meat in terms of quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms corresponded to 4.7×103±0.2, which is 14.5 times higher than in chilled meat samples and 8.4 times higher than in defrosted meat. In addition, the established value of the indicator of microbial contamination in re-defrosting meat exceeded the maximum permissible value established by regulatory enactments. Thus, there is a direct effect of repeated freezing of raw meat on its good quality and safety. Therefore, the solution to such a practical problem as identifying the thermal state of turkey meat acquires a significant role.
Keywords: Contamination, Meat hygiene, Meat spoilage, Microbiological control, Storage.

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**Research Paper**

## The effect of the pandemic on the consumption of animal products: the case of Kafkas university of Turkey

Aydin E and Ayvazoglu Demir P.

*Online J. Anim. Feed Res.,* 12(1): 37-45, 2022; pii: S222877012200006-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.6

Abstract
The COVID-19 pandemic has brought about some changes in consumption preferences and nutritional habits as well as lifestyles of individuals, such as education, working system, and social relationships, especially due to having to stay at home. In this study, the change in animal food consumption dispositions of students who had to stay at home during the pandemic and received online education was examined compared to the pre-pandemic period. In the study, since face-to-face teaching-learning activities were suspended, an online questionnaire was used to collect the study data from a total of 380 students registered at Kafkas University. The average monthly income of the participants and the share they allocated to the total food and animal products expenditures in the budget were determined as 539.64±21.00$, 132.73±5.08$, and 62.18±2.67$, respectively. Although the annual chicken meat consumption amount did not change according to the income levels of the students, it was determined that the consumption of animal products, such as beef, mutton-lamb, milk-yogurt, cheese, and eggs increased as the income level increased. During the pandemic period, the food consumption of 55.3% of the participants and the animal products consumption of 35.8% increased. On the other

hand, it was determined that the consumption of food and animal products increased as the income level increased during the pandemic compared to the pre-pandemic period. During the pandemic period, it was determined that red meat and salami-sausages consumption of 31.8% of the students decreased by 37.90% and 42.15%, respectively, but that the chicken meat consumption of 31.3% of the students and milk and dairy products consumption of 37.9% increased by 31.02% and 39.17%, respectively. As a result, it was determined that there were significant changes in the nutritional habits of the students during the COVID-19 pandemic, and it was determined that there were significant increases in the consumption of animal products other than red meat. The primary reason why red meat consumption did not increase is thought to be due to the high price of the product compared to consumers' income levels.
**Keywords**: Animal Products, Consumption, COVID-19, Meat, Pandemic.

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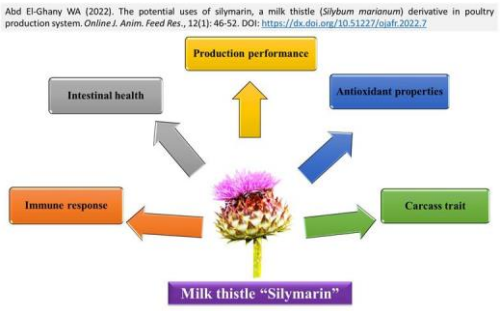
**Review**

## The potential uses of silymarin, a milk thistle (*Silybum marianum*) derivative in poultry production system

Abd El-Ghany WA.

*Online J. Anim. Feed Res.,* 12(1): 46-52, 2022; pii: S222877012200007-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.7




**Abstract**
Due to recent intensive poultry production, there is a great demand to use natural alternative feed additives. One of these alternatives is phytobiotics. Milk thistle (*Silybum marianum*) is a plant that has been used for many years as a natural remedy for the liver diseases. Silymarin is the major dried extract of milk thistle. Silymarin has many flavonolignans that showed antioxidant, anti-inflammatory, anti-fibrotic, anti-lipid peroxidative, immune stimulant, and hepatic cells stabilizing effects. In poultry production system, silymarin has been used in broilers as a growth promotor and in layers to improve the egg quantity and quality. It has been also used as a hepatotonic substance as a result of a potent antioxidant activity. The carcass trait showed improvement after treatment of broilers with silymarin. In addition, enhancement of the immune system and the intestinal health has been detected after application of silymarin in poultry diets. Accordingly, this review article aims to show the different potential uses of silymarin in poultry production system regarding its effect on production performance, antioxidant status, carcass traits, immune response, and intestinal health.
**Keywords**: Antioxidant, Carcass trait, Immunity, Intestinal health, *S. marianum*

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# PECAN SHELLING BY-PRODUCT AS A PARTIAL REPLACEMENT FOR CORN IN SWINE DIETS: PERFORMANCE, DIETARY ENERGETICS AND SAFETY ASSESSMENT

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*Supporting Information*

RESEARCH ARTICLE

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**ABSTRACT**: The objective of the present study was to evaluate the pecan shelling by-product (PSB) grindings as partial replacement for corn in swine diets and its exerting effect on sows' performance. Seventeen pregnant sows (210 kg of initial body weight) were used in a 14-d trial to evaluate the effect of partial replacement of corn with PSB on performance, dietary energetics, and safety. Treatments consisted of corn-soybean meal-based diet containing 0 or 10% of PSB in the diet, where the later replaced 0 or 16% of corn (as-fed basis). Ether extract (EE) content on PSB averaged 185 g/kg. Treatments including PSB had 1.9 times the EE compared to the treatment with only corn-soybean meal. Replacement of 16% of corn with PSB increased the estimated digestible (DE) and metabolizable energy (ME) values of the diet by 218 and 230 kcal/kg compared to control diet (90% DM basis), respectively. The higher predicted DE or ME intake for sow fed on PSB diet were 500 or 529 kcal/day compared to control diet, respectively; however, no differences on average daily gain (0.52 kg/d) between dietary treatments were observed. Neither was feed refusal for any dietary treatment nor difference on finishing their respective feed allowance. Sows consumed pecan shell at level of 0.75 g/kg of body weight daily, approximately. Intestinal activity was not affected by PSB consumption and fecal score averaged 3.0. Partial replacement of corn with PSB increased the estimated DE and ME for gestating diets by 7%, without influencing performance and intestinal function. The pecan shelling by-product supplementation at 10% inclusion level into a corn-soybean meal-based diet does not affect performance and its addition to the diet contributes to a more sustainable use of locally available feedstock in swine feeding.

**Keywords:** By-product, Corn-soybean meal, Pecan; Pigs, Sows diet.

## INTRODUCTION

Food production from livestock systems demands considerable resources such as land, water, and fuel. Additionally, this industry emits industrial contaminants that have long term ramifications unless actions are taken (Grossi et al., 2019). Livestock feeding accounts for roughly over 60 to 70% of total animal production cost (Zijlstra and Beltranena, 2013; Pomar and Remus, 2019) and rely heavily on commodities such as soybean and corn that are produced far from feeding operations (Wilfart et al., 2016, Mendes dos Reis et al., 2020). This has been the case over the last half century, as corn-soybean meal-based swine diet formulations promote better performance for growing and mature pigs compared to other cereal grains (Stein et al., 2016). The feed, food and fuel industry compete for corn which can jeopardize food security and social stability; this is especially important to Mexico where corn is a staple food (O'leary, 2016). Therefore, it is important to look for alternative feedstocks to reduce dependency on corn in feed formulations (Muscat et al., 2020). Across the world, local agro-food-industries generate several by-products that can be used in swine feeding (Zijlstra and Beltranena, 2013). The pecan shelling industry in northern Mexico generates by-products such as pecan shelling by-product grindings (PSB; "polvillo de nuez") which is rich in fat and polyphenol content (De la Rosa et al., 2011). The PSB chemical composition varies widely (Flores-Córdova, et al., 2016) which might be attributed to its parent feedstock composition variability and further amplified by processing related factors (Zijlstra and Beltranena, 2009). At present, PSB remains underutilized as a feedstock for animal feeding. A major research area is developing to evaluate its polyphenol components for biomedical purposes (Flores-Estrada et al., 2020). Based on its proximate components, the inclusion of PSB into swine diet formulations is expected to be comparable to the nutritional value of the replaced corn. Additionally, the inclusion of PSB in swine diets is expected to have a positive impact on feed and environmental cost. However, the suitability of PSB for swine diets is unknown. Hence, the objective of the present study was to evaluate the PSB as partial replacement for corn in swine diets and its exerting effect on sows' performance.



**Citation:** Buenabad L, Saenz E, Arce N, Gonzalez J, Martinez JA and Corral A (2022). Pecan shelling by-product as a partial replacement for corn in swine diets: performance, dietary energetics and safety assessment. *Online J. Anim. Feed Res.*, 12(1): 01-06. DOI: https://dx.doi.org/10.51227/ojafr.2022.1

## MATERIAL AND METHODS

### Animal handling, housing, and feeding

Seventeen pregnant sows (five gilts and twelve sows; York × Landrace × Duroc) were used to evaluate the effect of partial replacement of corn by pecan shelling by-product grindings (PSB) on sows' performance during midgestation (from d 53 to 67). Gilts were supplemented with altrenogest (Virbagest®, Virbac Mexico, S.A de C.V.) for oestrous synchronization and artificially inseminated with at least two doses of a semen of mature boar of proven fertility. Sows were bred using artificial insemination following oestrus detection after weaning. After pregnancy diagnosis, sows and gilts were randomly assigned to one of two dietary treatments. Sows were not re-grouped and kept in their same pen of origin (3 to 5 sows) to prevent changes in social ranking within each group. The sows used in this trial were cared for in accordance with the guidelines established in the Official Mexican Regulations on Animal Care (NOM-062-ZOO-1999, 2001). Experimental diets were based on corn-soybean meal supplemented with vitamins and minerals and formulated to meet or exceed nutrient requirements of pregnant sows (NRC, 2012). Dietary treatments (Table 1) were as follows: T1; corn-soybean meal diet (CTL) and T2; corn-soybean meal diet containing 10% of PSB, where the later replaced 16% of corn (as-fed basis). Pens were 54 $m^2$ with 36 $m^2$ overhead shade, nipple drinker, and 5 m concrete feeder bunk. Sows were fed 2.3 kg per sow once a day in the mornings (0800 h) and were allowed free access to drinking water. Initial and final body weights (BW) were recorded to calculate the average daily gain (ADG). Including a non-conventional feedstock into the diet can generate unintended consequences on intestinal activity such a diarrhea or constipation, thus every morning fecal score was monitored by pen for the whole period. Fecal score ranged from zero to five: 0, total absence of feces; 1, dry and hard; 2, between dry and soft; 3, soft but still formed; 4, soft and wet; 5, liquid feces (Oliviero et al., 2010).

### Sample analysis and Estimation of dietary energy

Pecan shelling by-product and feed samples were subject all or in part to the following analysis: DM (oven drying at 105 ºC until no further weigh lost; method 930.15; AOAC, 2000), ash (method 942.05, AOAC, 2000), Kjeldahl N (method 984.13, AOAC, 2000); crude fiber (method Ba 6a-05; AOCS, 2005) and ether extract (Thiex et al., 2003). Dietary digestible and metabolizable energy values were estimated using the values of dietary proximal components fed on prediction equation for growing pigs (Noblet and Perez, 1993), and subsequently the intermediate results were applied into a second prediction equation for sows (Noblet and Shi, 1993).

### Statistical design and analysis

The data from this trial was analyzed as a randomized block design experiment using PROC GLM procedure (SAS Inst. Inc., Cary NC; Version 9.1), considering parity for blocks, and sows as experimental unit according to the following statistical model: $Y_{ij}=\mu+\beta_i + T_j + E_{ij}$

Where $\mu$ is the common experimental effect; $\beta_i$ represents parity; $T_i$ represents the dietary treatment effect; $E_{ij}$ represents the residual error. Treatments effects were evaluated by means of t-test.

## RESULTS AND DISCUSSION

### Dietary nutrient content of pecan shelling by-product grindings

The pecan shelling industry's main product is half pecan kernels of different grading, but the industry also generates by-products such as shell and pecan shelling by-product grindings. The average PSB is composed of shattered pecan kernel pieces in combination with shelling grindings which are rich in fatty acids (Sevimli-Gur et al., 2021) and polyphenol content (De la Rosa et al., 2011), respectively. As expected, the analyzed ether extract (EE) content on PSB was high, averaging 185 g/kg (Table 2); the EE value of PSB was five times higher than that of tabulated fat values on replaced corn (as-fed basis; NRC 2012). It is well known that pecan fat and polyphenol content is highly variable, which is influenced by production year (Flores-Córdova et al., 2016), place of origin (De la Rosa et al., 2011), and cultivar (Cason et al., 2021). In this regard, the estimation of nutrient content on by-products such as PSB might be challenging and make it difficult to predict its influence on animal performance. In fact, the inclusion of PSB on backyard pig's diets led to unsuccessful animal performance. The basis of this is not certain, but the uncontrolled level of inclusion of this feedstock into the pig's diets plays a major role. The fat component in pecan nut contains roughly 65, 27, and 7% of monounsaturated, polyunsaturated, and saturated fatty acids, respectively (Rivera-Rangel et al., 2018). The consumption of residual fat in PSB seems energetically and metabolically important to improve sow´s performance on her current pregnancy (Metzler-Zebeli, 2021). However, polyphenols are considered "antinutritional factors" which influence feed palatability (Windisch et al., 2008; Huang et al., 2018; Caprarulo et al., 2021) and nutrient digestibility (Bravo, 1998). Conversely, it also exerts antiparasitic, antimicrobial and antioxidant activity (Huang et al., 2018). The high content of bioactive compounds with antimicrobial activity in PSB (Cason et al., 2021) leads us to assume that this is a suitable feedstock to replace conventional growth promotion additives in swine production (Huang et al., 2018; Flores-Estrada et al., 2020; Cason et al., 2021). Likewise, the antioxidant activity of polyphenols can help to counteract the reactive oxygen species (ROS) production during periods of stress (Flores-estrada et al., 2020), a common condition for sows reared under intense animal production setting (Agyekum and Nyachoti, 2017).



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**Table 1** - Ingredients and composition of experimental diets fed to gestating sows as is.

| Item | Control | PSB, 10% |
|---|---|---|
| Corn | 61.5 | 51.5 |
| Soybean meal | 10.0 | 10.0 |
| Wheat bran | 24.0 | 24.0 |
| Pecan shelling by-product grindings | 0.0 | 10.0 |
| Canola oil | 1.5 | 1.5 |
| Feed supplement | 3.0 | 3.0 |
| | 100.0 | 100.0 |
| **Analyzed composition** | | |
| Dry matter | 91.4 | 92.5 |
| Crude Protein | 15.7 | 16.3 |
| Crude fiber | 6.5 | 5.7 |
| Ether extract | 4.3 | 8.3 |
| Ash | 6.3 | 6.4 |
| **Estimated dietary energy** | | |
| Digestible energy, kcal.kg$^{-1}$ | 3334 | 3552 |
| Metabolizable energy, kcal.kg$^{-1}$ | 3110 | 3340 |

PSB = Pecan shelling by-product

**Table 2** – Proximate composition of pecan shelling by-product grindings (g.kg$^{-1}$ as-fed basis).

| Item | N | Mean | SD |
|---|---|---|---|
| Dry matter | 3 | 929.6 | 6.9 |
| Crude protein | 2 | 178.0 | 1.4 |
| Crude fiber | 1 | 230.1 | - |
| Ether extract | 2 | 184.8 | 6.2 |
| Ash | 3 | 16.6 | 0.4 |

**Estimated dietary energetics on gestating diets**

All nutrients are equally important for swine diet formulation; however amino acids, phosphorus, and energy are considered the most expensive components in swine diet formulations (Noblet and Perez, 1993; Velayudhan et al., 2015; Stein et al., 2016). In this regard, underutilized feedstock such as a good quality PSB could be a valuable non-conventional feed ingredient for swine diet formulations; this is especially true under the forecasted energetic conventional feedstuffs shortage (Velayudhan et al., 2015). Based on its proximate components, the estimated digestible energy (DE) values of dietary treatments were 3334 and 3552 kcal/kg (90% DM basis) for control and PSB supplemented diet, respectively (Table 1; Noblet and Perez, 1993; Noblet and Shi, 1993). Similarly, the estimated metabolizable energy (ME) values for control and PSB supplemented diet were 3110 and 3340 kcal/kg (90% DM basis), respectively (Noblet and Perez, 1993; Noblet and Shi, 1993). The higher EE content in PSB increased corresponding EE content in the tested dietary treatment, which was 1.9 times higher than that in control diet. However, the corresponding estimated dietary DE and ME on the PSB supplemented diet was just 7% higher compared to the control diet in both cases. The basis of this is not certain. As dietary EE content increases, its digestibility improves until fat content is greater than 80 g/kg of DM (Noblet and Shi, 1993) as in this case (89 g/kg of DM) which limits dietary energy utilization by the sow. One way to solve this issue could be to reduce or remove total supplemental vegetable oil in the diet itself to increase fat digestion from PSB which would reduce the feed cost even further.

**Non-conventional feedstock dietary supplementation on sows' performance**

Local agro-industrial by-products from fruits and vegetable processing around the world are gaining interest as an environmentally friendly strategy for animal feeding (Correddu et al., 2020). The aim is to reduce dependency on commodities shipped long distances to reduce carbon footprint of transportation (De Quelen, et al., 2021). Commodities such as corn and soybean meal have a substantial influence on cost of diet formulations even at levels of inclusion as low as 10% (Wilfart et al., 2016). In close agreement with the estimated dietary DE or ME values on dietary treatments, average daily gain in sows was not different between treatment groups, which averaged 0.52 kg/d (Table 3). This is within normal range values for sows reared under similar conditions (Deng et al., 2021). However, sows on the PSB treatment had numerically greater average daily gain (29%) compared to those on the control diet. The last is consistent with the slight improvements on DE and ME on the PSB supplemented diet. The high variation on ADG within groups is ascribed to the difference in feed intake. This is the result of hierarchical system within each group of pigs where low-ranking sows



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are in disadvantage compared to high-ranking sows at feeding time (Bench et al., 2013). Group housing gestating sows aimed to improve sows´ welfare (Norring et al., 2018). With legislation moving away from the use of stalls (Greenwood et al., 2014), figuring out how to feed sows on forecasted production scenarios is important (Deng et al., 2019).

Table 3 - Treatment effects on performance of gestating sows.

| Experimental groups / Response variable | Control | PSB, 10% | SEM | P-Value |
|---|---|---|---|---|
| Body weight at 53 d of gestation, kg | 210.9 | 210.4 | 8.742 | >0.10 |
| Body weight at 67 d of gestation, kg | 217.2 | 218.6 | 8.669 | >0.10 |
| Average daily gain, kg | 0.45 | 0.58 | 0.162 | >0.10 |
| Fecal score | 3.0 | 2.9 | 0.035 | >0.10 |

PSB 10% = Dietary treatment supplemented with 10% pecan shelling by-product

**General safety assessment of pecan shelling by-product fed on swine**

As a non-conventional feedstock, PSB presents some challenges that need to be addressed to safely recommend its inclusion in feed formulations. The safety assessment starts by completing a comparative evaluation of candidate feedstock (pecan shelling by-product; PSB) with a comparable feed (corn) that has a known history of safe use in animal feeding (Glenn, 2008). As described, there were no issues on sows' performance when they were fed a PSB supplemented diet. Gestating sows are fed to meet or exceed their nutritional requirements but not to the point of meeting satiety (Greenwood et al., 2019). In this management system, gestating sows are less selective of unpalatable feed (Agyekum and Nyachoti, 2017). In this trial, sows fed the PSB supplemented diet did not show any refusal from their feed allowance, even when the tested diet was high on polyphenol content. No differences (P>0.05) were observed between treatment groups for time spent eating. Conversely, lactating sows fed *ad libitum* took longer to finish their feed allowance when PSB was included at levels as low as 2.5% of the diet (as-fed basis; unpublished data). Constipation is a recurrent problem in sows which is influenced by level of feed and water intake, diet composition and their interactions between them with the intestinal microbiota (Pearodwong et al., 2016). Unattended constipation can lead to poor performance around farrowing and during lactation (Oliviero et al., 2010). Fecal score was not different between treatment groups which averaged 2.9 and 3.0 for PSB supplemented and control diet, respectively. Based on average feed intake and PSB composition, sows consumed approximately 0.75 g/kg of BW of pecan shell which corresponded to 14% of pecan shell supplemented to rats where the maximum safe consumption recommendation for humans came from (Dolan et al., 2016).

## CONCLUSIONS

Partial replacement of corn with pecan shelling by-product grindings increased the estimated DE and ME for gestating diet by 7%; however, it did not influence sows' performance at mid-gestation period. Based on the estimated dietary energetics, the PSB energy value seems to be comparable to that of corn for mature pigs. Furthermore, no clinical signs of health problems were seen during the trail. In agreement with sows' performance, estimated dietary energetics, and corn use reduction (16%), PSB can be included into sows' diet at the 10% level when replacing corn. This feeding strategy can contribute to the sustainable use of locally available feedstock in swine feeding, without affecting their productive performance.

## DECLARATIONS

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**Conflict of interest**
The authors declare no conflict of interest.

**Authors' contributions**
Buenabad L. conceived and designed the study, reviewed the manuscript, and gave final approval of the submitted version of this manuscript. Saenz E. participated with data acquisition, analyzed, and interpreted the data. Arce N., Gonzalez J., and Corral A. analyzed and interpreted the data, and edited the manuscript for important intellectual contents. Martinez J.A. participated with data acquisition and critically revised the manuscript for improvements.


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# EFFECT OF A NEW COMPLEX MYCOTOXIN ADSORBENT ON GROWTH PERFORMANCE, AND SERUM LEVELS OF RETINOL, TOCOPHEROL AND 25-HYDROXYCHOLECALCIFEROL IN PIGS FED ON MYCOTOXIN-CONTAMINATED FEED

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*Supporting Information*

RESEARCH ARTICLE

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**ABSTRACT**: The objective of the present study was to investigate the effects of commercial analogue mycotoxin adsorbent (CAMA) and Gepasorbex as a new complex mycotoxin-adsorbent additive on growth performance and serum retinol, tocopherol and 25-hydroxycholecalciferol concentrations of fattening young pigs (n=90), which fed on the combined feed contaminated with mycotoxins. Animals were randomly allocated to 3 groups with 30 heads in each: the first group of pigs (control) fed the basic diet in grower and finisher periods; the second group fed the basic diet with the 0.15% by weight of feed of the mycotoxins-adsorbent as commercial analogue; the third group fed the basic diet with the addition of 0.15% by weight of feed of Gepasorbex, a new compound of mycotoxin-adsorbent additive. On the 48th and 88th days of fattening, blood samples were taken from the pigs for testing the vitamins A, E and 25-hydroxycholecalciferol levels. Piglets from third experimental group followed by group 2 animals showed better growth rates and significantly ($P<0.001$, $P<0.01$, respectively) exceeded analogues from the control group in live weight and average daily gain in all age periods which showed a noticeable positive effect of diet's mycotoxin adsorbents, especially Gepasorbex, on animal growth rates. The results of the laboratory study showed that in the pigs from third experimental group, which fed on Gepasorbex with compound feed, the serum concentrations of retinol, tocopherol and 25-hydroxycholecalciferol were within the biological reference interval and significantly exceeded ($P<0.05$) similar indicators from the control group in both periods. The results of the experiment showed that when animals are subjected to diets contaminated by mycotoxins, use of a new complex action preparation like Gepasorbex can mitigate the negative impacts of mycotoxins on animal performance, and is more capable to remove mycotoxins without binding to dietary vitamins.

**Keywords:** Gepasorbex, Mycotoxins, Retinol, Tocopherol, 25-hydroxycholecalciferol.

## INTRODUCTION

It is known that the use of intensively innovative technologies and pigs of high genetic potential in order to ensure the productivity through efficient use of feed resources, maximum preservation of animals and prevention of various diseases is a feature of the modern pig industry (Caisin et al., 2011). This fact places significant demands on the provision of quality and environmentally friendly feed, which is associated with their contamination by various toxins, heavy metals, pesticides, nitrates, etc. (Bryden, 2012; Holanda et al., 2021). In commercial pig farms, the presence of mycotoxins in feed is, unfortunately, quite common. Therefore, various measures are used to prevent pigs' diseases caused by mycotoxins, as well as to reduce economic damage (Kanora and Maes, 2009). It should be noted that mycotoxins are toxic secondary metabolites formed by various fungi, such as *Aspergillus*, *Penicillium* and *Fusarium*, which can contaminate feeds and, consequently, food (Pierron et al., 2016; Conte et al., 2020; Hussain et al., 2020; Ulrikh and Smolovskaya, 2021).

Despite improvements in good agricultural and industrial practices, the mycotoxin contamination is unavoidable, and contaminants occur almost everywhere in varying concentrations and ratios in the diet of both animals and humans (Ramos and Hernandez, 1997; Bryden, 2012). Global climate change has resulted in unusual weather patterns, with increased frequency of drought, flooding and temperature extremes (Godde at al., 2021). These changes in weather all increase the chance of mycotoxin contamination of feed grains. Increased global trading of feed grains also increases the chance that blends of grains will result in combinations of different mycotoxins in a diet of animals (Mullan, 2017).

Currently, scientists from the world's best laboratories have isolated more than 300 to 400 mycotoxins with their laboratory identification of about 20 species (Pereira at al., 2019). Therefore, it was found that mycotoxins are a group of



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substances with different structure, which is a secondary metabolite of toxicogenic fungi. According to experts, 25% of world grain production shows contamination with mycotoxins (CAST, 2003; Eskola at al., 2019). In addition, these substances are involved in a number of toxic mechanisms, in particular: disruption of several metabolic functions in both humans and animals. Mycotoxicosis is common in pig breeding because pigs are quite sensitive to mycotoxins, where the latter affect the reduction of feed intake, the development of diseases of the reproductive organs, reproductive dysfunction, weakening the body's immune system, reducing resistance to diseases (coccidiosis, colibacillosis, etc.), the cost of preventive and veterinary measures, reducing the effectiveness of vaccines and drugs (D'Mello et al., 1999; Dersjant-Li et al., 2003). However, as noted the susceptibility of pigs to mycotoxicosis is influenced by a number of factors, such as: sex and age, and each mycotoxin has its own mechanism of action with specific clinical manifestations, according to the accepted amount (Cote et al., 1985; Roger and Coulombe, 1993; Hussein and Brasel, 2001). As a result, the losses from pig mycotoxicosis are considerable: a marked decrease in productivity, low reproductive capacity, high mortality, forced slaughter of pigs, which causes significant economic losses (Reddy et al., 2018). The parameters of the impact of mycotoxins on pigs are suppression of immune status, hepatotoxic and nephrotoxic effects, low consumption or complete refusal of feed, increased feed consumption, reduced live weight gain, gastrointestinal and cardiovascular disorders, high mortality, etc. (Holanda and Kim, 2020). It is unfortunate that the body does not produce antibodies to mycotoxins (Díaz-Llano and Smith, 2007).

In order to prevent mycotoxicosis in pigs, world scientists and practitioners have developed ways to minimize the action of mycotoxins aimed at removing mycotoxins with various mineral and organic adsorbents (Ramos and Hernandez, 1996; Huwig et al., 2001; Battacone et al., 2007; Holanda et al., 2021). Studies by a number of authors have shown that long-term use of sorbents revealed a decrease in the content of vitamins A, D and E in the blood of animals (Lawson et al., 1971; Harvey et al., 1994; Kihal et al., 2022).

Therefore, a number of studies are currently being conducted to find the most effective sorbents that will get rid of mycotoxins and preserve vitamins in animals. Due to the urgency of the problem, the study aimed to determine the effectiveness of use of a new complex mycotoxin-adsorbent additive Gepasorbex a product of VetServiceProduct in combined feeds contaminated with mycotoxins, to increase the productivity of pigs.

## MATERIALS AND METHODS

### Ethical regulation

The rules for the treatment of animals in the experiment were fully complied with European legislation on animal protection and comfort kept on farms (Directive № 95/58 EU «From the protection of farm animals» of the EU Council of 20.07.1998 as amended by EU Regulation № 806/203 of 14.04.2003, № 91/630 EU «Minimum standards for the protection of pigs» of 19.11.1991 as amended by EU Regulation). The protocol of experimental study on blood sampling in pigs, approved by the local Commission on Bioethics of the National University of Life and Environmental Sciences of Ukraine on Good Clinical Practice (GCP) for the protection and humane treatment of experimental animals.

### Experimental design

A total of 90 heads fattening young pigs were used in the experiment, where the maternal form was a combination of the Large White × Landrace breeds, and the paternal form was boars of the Maxter terminal line. The animals were housed on the commercial farm of the Tavriya Pigs Limited Liability Company placed in Skadovsk district, Kherson region, Ukraine. Animals was divided into two grower and finisher periods: Grower period was included of animals (12-17 weeks old) with a live weight of 30-60 kg consumed 2.4-2.6 kg feed per head per day using the following grower feed type of nutritional value: crude protein = 166.7 g/kg and exchange energy = 13.562 MJ/kg. The pigs were placed on a concrete slotted floor with an area of 0.65 $m^2$/head. Finisher period of fattening was included of animals with a live weight of 61-100 kg (17-22 weeks old) consumed 2.8-3.0 kg feed per head per day using a combined feed type of nutritional value with 146.7 g/kg crude protein and 13.411 MJ/kg exchange energy. The pigs were placed on a concrete slotted floor with an area of 0.85 $m^2$/head.

### Feeding

As the basic diet (BD) it was used combined feed of own production for the use of premixes of production produced by Alternativa LLC. When transferring pigs from the rearing shop to the fattening shop of the first period, in order to equalize the animals and purity of research in the period from 11-12 weeks, the equalization period started. Then all experimental animals were divided into three groups (on the principle of analogues) of 30 heads: the control group of pigs fed the BD of both grower and finisher»; pigs of the second experimental group consumed the BD of both grower and finisher with the addition of 0.15% by weight of feed CAMA; and the third experimental group fed the BD of both grower and finisher with the addition of 0.15% by weight of feed complex preparation of Gepasorbex (Table 1).

The composition of 1 kg of Gepasorbex produced by VetServiceProduct LLC contains the following active ingredients (%): silica dioxide (60.2-70.8); aluminum oxide (8.0-12.0); magnesium carbonate (1.0-2.5); titanium dioxide (0.8-0.15); selenium (0.32-0.35); clineopleolite (4.2-4.5); active fodder yeast (8.0-10.0); milk thistle *Silybum marianum* (18.0-20.0) (the registration certificate = AB-08268-04-19).



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The main feed used for feeding pigs of the experimental groups according to laboratory studies was recognized as slightly toxic to aflatoxin B1 (≤ 0,05 μg/kg), ochratoxins (≤ 0.1-0.4 μg/kg) and zearalenone (≤ 0.25 μg/kg), contract №837 from 06.07.2021 (Expert Center of Diagnostics and Laboratory Support «Biolights» LLC, Kyiv region, Ukraine. In the experiment, fattening indexes were studied according to the methods (Ibatullin et al., 2017).

**Table 1** - The scheme of the experiment

| Age | Group | Feeding conditions |
|---|---|---|
| Age 11-12 weeks - EW | | |
| Age 12-17 weeks | 1st, Control | BD for grower |
| | 2nd, Experimental | BD + 0.15% by weight of feed CAMA |
| | 3rd, Experimental | BD + 0.15% by weight of feed Gepasorbex |
| Age 17-22 weeks | 1st, Control | BD for finisher |
| | 2nd, Experimental | BD + 0.15% by weight of feed CAMA |
| | 3rd, Experimental | BD + 0.15% by weight of feed Gepasorbex |

EW= equalization period; BD= basic diet; CAMA= commercial analogue mycotoxin adsorbent.

**Serum retinol, tocopherol and 25-hydroxycholecalciferol measurement**

At 12, 14, 17 and 22 weeks, live weight (in kg) and average daily gain (in g) were measured. On the 48th and 88th days, 30 blood samples were taken from pigs of the experimental groups, on an empty stomach in the morning by puncture of the jugular vein to determine the contents of retinol, tocopherol and 25-hydroxycholecalciferol. Serum samples for the content of these vitamins were conducted in the independent laboratory of Expert Center of Diagnostics and Laboratory Support «Biolights» LLC, Kyiv. Vitamins A and E were examined by high performance liquid chromatography, analyzer and Agilent 1200 HPLC System with UV-detector; Recipe complete Kit (Germany), the detection wavelength for the determination of vitamin A was 328 nm and for vitamin E it was 286 nm. The flow rate was 0.750 ml/min., the temperature of the column thermostat + 30.0°C, and 25-hydroxycholecalciferol measured by electrochemiluminescence immunoassay (ECLIA) method, Cobes e 601 analyzer, Roche Diagnostics system (Germany).

**Statistical analysis**

Data were analyzed using Statistica 12.0 (StatSoft Inc., 2014, www.statsoft.com). Results are presented as mean ± standard deviation (X ± SD). The following significance levels were used for the study: P <0.05; 0.01 and 0.001.

## RESULTS AND DISCUSSION

A significant difference in productive traits (live weight and average daily gain) of pigs of the control and experimental groups was observed at the age of 56 days, or at 14 weeks (Table 2). It should be noted that all piglets had a live weight of 33-34 kg when put to fattening. During 14-weeks piglets from third experimental group fed on Gepasorbex significantly (P<0.05) by 1.93 kg in live weight exceeded from the control group; for average daily gain significantly (P<0.001) exceeded by 114.3 g from the control group and by 50 g (P<0.05) over the second group fed CAMA. Regarding the 17th weeks: the animals of the third experimental group significantly (P<0.05) exceeded the live weight by 2.3 kg over the piglets of the second experimental group and by 3.63 kg (P<0.001) over to the analogues of the control group. In the piglets of the control and second experimental groups, the average daily weight gain was significantly lower by 81.0 g (P<0.001) and 38.1 g (P<0.05), respectively, than in pigs of the third experimental group. At the age of 22 weeks of fattening, young pigs of the second and third experimental groups had a significantly advantage in live weight by 2.47 kg (P<0.01), 5.10 kg (P<0.001) and average daily gain by 32.36 g (P<0.05), 41.90 g (P<0.01) over control group.

**Table 2** - Productive traits of experimental groups of pigs

| Parameters / Group / Age, (n = 30) | | 1st, control | 2nd, experimental | 3rd, experimental |
|---|---|---|---|---|
| 12 weeks | Live weight, kg | 35.50±0.717 | 35.03±0.812 | 35.83±0.649 |
| 14 weeks | Live weight, kg | 45.80±0.637 | 46.23±0.768 | 47.73±0.629* |
| | Average daily gain, g | 735.7±15.75 | 800.0±19.19** | 850.0±12.04***a |
| 17 weeks | Live weight, kg | 62.87±0.610 | 64.20±0.791 | 66.50±0.645***a |
| | Average daily gain, g | 812.7±15.64 | 855.6±10.33* | 893.7±8.45***b |
| 22 weeks | Live weight, kg | 93.33±0.471 | 95.80±0.720** | 98.43±0.544***b |
| | Average daily gain, g | 870.5±11.92 | 902.86±9.55* | 912.40±8.55** |

N= number; Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (in comparison with animals of the first control group); a= P<0.05; b= P<0.01 (in comparison of animals of third experimental group with analogues of second experimental group).



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**Table 3 -** Dynamics of the content of vitamins in the blood serum of pigs

| The name of vitamins, unit | Group, (n = 10) / vitamin content | | | Biological reference interval |
|---|---|---|---|---|
| | 1st, control | 2nd, experimental | 3rd, experimental | |
| **Age of pigs – 12 weeks (48 days)** | | | | |
| Retinol (vitamin A), μg/mL | 25.16±1.28 | 25.84±1.39 | 27.12±1.65 | 27.0-30.0 |
| Tocopherol (vitamin E), μg/mL | 3.76±0.52 | 4.28±0.39 | 5.71±0.96* | 5.7-6.4 |
| 25-hydroxycholecalciferol (vitamin D), ng/mL | 25.42±1.54 | 25.75±1.38 | 31.05±2.12* | 30.0-32.0 |
| **Age of pigs – 22 weeks (88 days)** | | | | |
| Retinol (vitamin A), μg/mL | 36.13±1.82 | 42.29±1.67* | 52.88±1.95*** | 50.0-60.0 |
| Tocopherol (vitamin E), μg/mL | 5.02±0.37 | 5.64±0.29 | 6.62±0.54* | 6.5-6.8 |
| 25-hydroxycholecalciferol (vitamin D), ng/mL | 27.24±0.87 | 28.15±0.92 | 30.89±1.14* | 30.0-32.0 |

N= number; significant: *= P <0.05; ***= P <0.001.

Of the several authors that the use of feeds with the addition of mycotoxin adsorbents in groups of animals improved their growth characteristics (Huwig et al., 2001; Duan et al., 2014; Patience et al., 2014; Weaver et al., 2014; Frobose et al., 2017). Noted that the use of phytobiotics with adsorbent of mycotoxin to mitigate the negative effects of multiple mycotoxins in pig diets increased their productivity, improved the absorption of feed elements and had better biochemical parameters of hepatic metabolism and immune status of pigs (Holanda et al., 2021)

It should be noted that adsorbents of mycotoxins differ from each other and from generation to generation due to technological developments are becoming more sophisticated and diverse in adsorption properties, as well as show an indirect therapeutic effect. Feed sorbents have the ability to quickly bind a wide range of toxicants. Sorbents are stable at different pH values, thermostable during feed granulation. The use of mycotoxin adsorbents as feed additives is beneficial for reducing the toxic effects of mycotoxins in pigs, which provides a more sustainable use of feed. There are many mechanisms by which adsorbents mitigate the toxic effects of mycotoxins in feed, one of which is adsorption when the mycotoxin interacts with another molecule (adsorbent) and is not absorbed by animals (Boudergue et al., 2009).

In the adsorbed form, the mycotoxin will be excreted in the feces, and its toxic effects will be minimized in animals. The next mechanism is the use of these agents to strengthen the immune function and intestinal health of the animal, such agents often include the use of prebiotics, probiotics, postbiotics, phytobiotics and synbiotics (Holanda and Kim, 2020).

However, many feed additives with sorption properties bind vitamins, macro- and micronutrients (Papaioannou et al., 2002; Kihal et al., 2021). According to the results of studies by other authors, it has been proven that long-term use of sorbents revealed a decrease in the content of vitamins A, D and E in blood of animals and poultry. Aflatoxin-B1 caused detrimental effects on liver health and electrolyte balance in pigs, leading to impaired liver function and structure of the liver and kidneys (Schell et al., 1993). The productivity and resistance of piglets depends on providing them with sufficient nutrients and biologically active substances. The latter include vitamins A, D and E, which ensure the normal course of biochemical and physiological processes in the body, have an impact on the growth and development of animals (Kihal et al., 2022).

As a result of experimental studies, it was found out a decrease in the content of some vitamins in the pigs of control and second experimental groups over animals of third experimental group (Table 3). On 48th and 88th days, in the pigs of the third experimental group, serum concentrations of retinol (27.12 μg/mL and 52.88 μg/mL), tocopherol (5.71 μg/mL and 6.62 μg/mL), 25-hydroxycholecalciferol (31.05 ng/mL and 30.89 ng/mL) respectively, detected within the biological reference interval. This indicates the effect of Gepasorbex on the relative bioavailability of these vitamins in the body of pigs third experimental group.

On the 48th and 88th days, the piglets of the second experimental group fed CAMA relative to the minimum value of the biological reference interval reduced serum concentrations, %: retinol (↓4.3 and ↓15.4), tocopherol (↓24.9 and ↓13.2), 25-hydroxycholecalciferol (↓14.2 and ↓6.2). A similar trend was noted in the animals of the control group on the 48th and 88th days - a decreased of serum retinol (↓6.8% and ↓27.7%), tocopherol (↓34.0% and ↓22.8%), 25-hydroxycholecalciferol (↓15.3% and ↓9.2%) relative to the minimum value of the biological interval.

Given that retinol, in addition to its antioxidant function, stimulates the growth of connective tissue («growth vitamin»), its deficiency often reduces weight gain (see Table 2). It is postulated that the decrease in vitamin A levels in the liver is the result of the consumption of T-2 toxin (Dvorska and Surai, 2001) and, as a consequence, a decrease in intestinal absorption of fat-soluble nutrients. Hoehler et al. (1996) suggested that mycotoxins, by stimulating lipid peroxidation of intestinal enterocytes, lead to the damage that significantly contributes to the disruption of retinol absorption. In our studies, this clearly observed in the pigs of control group.

In turn, the researchers noted that the presence of ochratoxin A in the diet significantly reduced the concentration of



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α-tocopherol in the liver. In addition, aflatoxin in pig diets reduced serum tocopherol and retinol concentration compared to control and pre-test values and decreased tocopherol concentration in cardiac tissue, which was recorded with animals of control group in the experiment (Harvey et al., 1994). Similar results were obtained by Ponchon et al. (1969), Lawson et al. (1971) on the negative effect of feed mycotoxins in animal diets on the inhibition of cholesterol synthesis, and subsequently on the activation of vitamin D, calcium and phosphorus balance in pigs.

## CONCLUSION

The results of the experiment show that a new complexes mycotoxin adsorbent a positive influence on the growth performance of piglets, increasing the bioavailability of retinol, tocopherol and 25-hydroxycholecalciferol in the body of pigs. The recommended rate a new complexes mycotoxin adsorbent for young fattening pigs is 0.15% by weight of feed. This result can be used in pig farming and also there is a need to study a feed costs and economic efficiency of using a new complexes mycotoxin adsorbent.

## DECLARATIONS


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### Authors' contribution

F.Rostyslav and L.Vadym participated in the design of study, performed the experiments and writing original manuscript. L.Anna, Sh.Mykola and L.Leonid assisted in performing the study, data arranging, and calculation. L.Vadym and L.Anna critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.


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### Conflict of interests

The authors have not declared any conflict of interests.

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# COMPARATIVE EVALUATION OF PHENOTYPIC RANKING DECISIONS AND TRAIT PREFERENCES OF SHEEP PRODUCERS IN AMHARA REGIONAL STATE OF ETHIOPIA

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**ABSTRACT**: This study was aimed to investigate comparative trait preferences of farmers' and selection practices for Rutana and Gumuz sheep breeds in existing production system. Ranking method, such as direct and own-flock ranking experiment with sheep keepers were carried in smallholder and large-scale production systems. Appearance, coat colour and fast growth rate were important traits in selecting breeding rams in smallholder system where as fast growth rate, tail length and appearance were preferred in large-scale system. Mothering ability, multiple births and lambing interval were most preferred for ranking breeding ewes in both systems. The mean of all objectively measured body conformation traits and body weight varies significantly across the farmers ranking categories and in line with their selection decision. In Gumuz sheep breed the average body weight of ewes which ranked as best was superior to rank as poor (34.5 kg vs. 26.7kg); whereas, in Rutana breed it was 39.4 kg vs. 29.5 kg ($P<0.05$). The farmers' breeding objectives were improving reproduction, conformation and growth traits, which can increase net cash income per flock through increased number of marketable animals for meat production. Therefore, considering meat production traits is recommended as feasible strategy for future for Gumuz and Rutana sheep genetic improvement and conservation program.

**Keywords:** Breed improvement; Conservation; Gumuz sheep; Ranking experiment; Rutana sheep.

## INTRODUCTION

Sheep productions in developing countries like Ethiopia is an important livestock farming activity and contributed immensely to the subsistence, economic and social livelihood of the smallholder farmers, in terms of generating income, meat, milk, skin and fiber (Hirpa and Abebe, 2008; Adem et al., 2018). Sheep production in developing countries is largely based on traditional breeds and characterized by diverse and multiple farmer breeding objectives (Solomon et al., 2008; Laouadi et al., 2018; Haile et al., 2019).

In Ethiopia, there are nine sheep breeds (Solomon et al., 2008) and 40 million sheep population (Central Statistical Agency of Ethiopia, 2020), which is distributed throughout the country. Despite the diverse sheep breeds in Ethiopia, the productivity and the contribution of sheep to the livelihood of resource poor farmers and the country economy is far below the potential. This might be attributed to the lack of appropriate indigenous breed improvement and utilization strategies (Solomon et al., 2011).

The local Gumuz sheep breed is prolific, adaptable to hot environmental condition and known for their tasty meat (Solomon et al., 2011). Previous study revealed that Gumuz sheep had diseases tolerance ability with a better survival rate compared with Rutana sheep, which is exist in the same area. However, it has been ranked poorly for its growth and body weight compared to the Rutana sheep (Solomon et al., 2008). Rutana sheep was introduced in the north western lowlands for crossbreeding with Gumuz as the breed is more preferable in the market and fetched higher price in the export market. Although, Gumuz sheep were preferred in terms of prolificacy and adaptation to the existing environment over Rutana and their crosses, the population of Gumuz sheep is considered decreasing in the study areas. As a consequence, the adapted local genetic resource of Gumuz breed which is the only thin-tailed breed of Ethiopia is losing its genetic diversity and considered to be declined (Solomon et al., 2008). A good understanding of production and breeding practices is fundamental to design a sound breeding program, which leads to sustainable utilization and conservation of the genetic resources (Hagos et al., 2018). Therefore, elucidating the updated comparative trait preferences of the local farmers on the two sheep breeds in the existed production systems has a paramount importance for designing effective breeding program. Breeding objectives and trait preferences can be identified through participatory approaches as advised by multiple scholars (Duguma et al., 2011; Königet al., 2016). Hence, the objective of the study was to identify the trait preferences and evaluating the selection decisions for Gumuz and Rutana sheep breeds under existing production system.



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## MATERIALS AND METHODS

### Study area description

The study was carried out in Metema and Quara districts of North Western Amhara regional stats, Ethiopia. The areas have an altitude range of 550-1680 m.a.s.l, mean range temperature of 22-43$^0$C and mean range annual rain fall of 850-1100mm (Solomon, 2007). Based on the number of sheep maintained and flocking practice, sheep production in the area classified as smallholder and large-scale producers. Therefore, those who keep below 20 sheep in free grazing with family labor is considered as smallholder and owners that keep above 20 up to hundreds of heads of sheep in ranch-based system by using hired labor is large scale producers.

### Study approach

Among the recommended participatory tools, direct ranking and own flock ranking experiment adopted from (Gemeda et al., 2010) were used to identify farmers trait preferences and selection decisions. For direct ranking a total of 240 sheep owners were randomly selected (180 from smallholder and 60 from large scale system) and used. Attributes for ewes and rams used in the own flock ranking were identified through an in-depth interview and discussion with respondents to rank the attributes in order of importance. To evaluate the selection decision of farmers, own flock ranking experiment on 40 randomly selected smallholder sheep producers and 20 large-scale were considered. Sheep owners were requested to classify their sheep into breed groups mainly based on morphology and coat colour. Within the two breed groups (Gumuz and Rutana) each owner was asked to select three ewes and rams which had lambed at least once in their flocks. Then each farmer asked to choose their best, average and poor-quality ewes and rams among the breeding ewes/rams in their flocks according to their opinion. Each owner provided at least three reasons in order of importance for the ranking ewes/rams and life history of the ranked animals were inquired and recorded. Weighting of reasons for given sets of preference was done in accordance to the farmers' ranking of importance of traits and in a similar way as done by Tadelle et al.(2012). The live body weight and some linear body measurements of the ranked animals were also taken.

### Data analysis

The statistical software SPSS Ver.19 (SPSS, 2010) was used to analyze the data from participatory identification trait preferences. Owner's preference rankings were summarized into index as weighted averages. Indices were calculated for ranked variables (selection criteria). Index was computed using the following formula as suggested by Kosgey et al. (2008). Sum of (3x for rank 1 + 2x for rank 2 + 1x for rank 3) given for a given reason divided by the sum of (3x for rank 1 + 2x for rank 2 + 1x for rank 3) for overall reasons. Body condition score (BSC) was done for the experimental sheep. As indicated by Yohannes et al. (2018) a scored between 1 and 5, where 1 = very thin; 2 = thin, 3 = medium, 4 = fat, 5 = very fat was used. The body weight and other linear body measurements were analyzed by the general linear model (GLM) procedure of SAS (2009). Weighted ranking methodology was applied for choosing between alternatives with multiple attributes in decision-making. Reasons 1-3 and sum of weighted ranks (Sum) and relative weights (Rel.weights) as proportion of the total Sum by breed and system adopted from (Bangweon and Seokjoong, 2016).

## RESULTS AND DISCUSSION

### Selection criteria for ranking of breeding ram

The farmers' selection criteria for breeding rams are presented in Table 1. Perusal of these results showed that first four selection criteria for rams, in descending order, were body conformation, coat colour, growth rate and tail length under small holder system whereas body conformation, growth rate and coat colour / tail length under large scale system. In both smallholder and large-scale production system, conformation trait receives high emphasis for ranking of breeding rams for both breeds.The principal reason why smallholder farmers ranked body conformation as first criteria to select a breeding ram was possibly their belief that well conformed ram showed good breeding potential and fetched better sale price in the market. Ranked traits for selection of breeding ram in this study was similar with earlier results of Solomon et al. (2010) and Nigussieet al. (2015) for indigenous sheep in central and Eastern Ethiopia. Next to body conformation smallholder farmers give high attention to colour as selection criteria for breeding ram. Hence, males with red and patchy of red and white colours were selected. This may due to preference of white or red colored in high demand for religious festival and have high local market values in comparison with pure black or unwanted coat colour. A previous study by Edea et al. (2012) on Bonga and Horro sheep breeds confirmed that beauty traits like coat colour might be associated with socio-cultural practices and market demand. At the same time, Nigussieet al. (2015) also reported that coat colour was the second ram ranking trait in crop-livestock production system for indigenous sheep breed in Eastern Ethiopia. In large scale production system, growth rate of lambs was the second selection criteria for ranking of best rams in both breeds. The reason why growth trait was used in large scale system is related with the producer's primary aim of production and level of awareness. This means that large scale producers are more commercial and market oriented than small scale producers that kept sheep for their subsistence. Therefore, large scale producers mainly targeted to utilize the



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border advantage and sale sheep to Sudanese traders who prefer a yearling male lamb. Hence, large scale produces needs to reared lambs, which can reach market weight sooner. Similar findings were reported by Nugussieet al. (2015) and Abebeet al. (2020) for indigenous sheep in Ethiopia, and Sheriff et al. (2021) for Arab and Oromo goat keepers in north western Ethiopia. According to their justification, the high attribute of fast growth of lambs and kids for breeding and meat production might due to high market demand and the proximity of the study areas to export market.

**Table 1** - Selection criteria for ranking of breeding rams in smallholder and large-scale system

| Selection Criteria | Smallholder system | | | | Large scale system | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | I | R1 | R2 | R3 | I | I |
| Body conformation | 44.4 | 17.2 | 21.7 | 0.32 | 15.0 | 26.7 | 13.3 | 0.19 | 0.26 |
| Coat color | 9.4 | 30.6 | 35.0 | 0.21 | 13.3 | 10.0 | 25.0 | 0.14 | 0.17 |
| Growth rate | 22.8 | 16.1 | 7.8 | 0.18 | 26.7 | 20.0 | 21.7 | 0.24 | 0.21 |
| Libido | 5.6 | 15.0 | 7.2 | 0.09 | 13.3 | 16.7 | 13.3 | 0.14 | 0.11 |
| Age at 1st mating | 3.9 | 8.3 | 10.0 | 0.06 | 10.0 | 8.3 | 10.0 | 0.09 | 0.08 |
| Tail length | 13.9 | 12.8 | 18.3 | 0.14 | 21.7 | 18.3 | 16.7 | 0.20 | 0.17 |

R = Rank; I = Index

**Selection criteria for ranking of breeding ewes**

As indicated in Table 2, body conformation was reported as the first selection criteria of breeding ewes in both production systems. Multiple births, mothering ability and lambing interval were ranked descending as the next three selection criteria of breeding ewes in large scale production system. Whereas mothering ability, multiple births and coat color were orderly ranked in smallholder production system. Previous study reported that mothering ability increases the chance of survival of young goats (Snyman, 2010; Tesema et al., 2020) and mainly given high emphasis by farmers in selecting breeding ewes, which is in support of the current study. The main reason why sheep farmers selecting ewes with good mothering abilities is by considering of the caring and nourishing potential of ewes for better growth and survival of lambs. This result is in agreement with Dugumaet al. (2011) reported that there was a high choice preference for good mothering ability of ewes in four indigenous sheep breeds of Ethiopia by anticipating a healthy and good-sized lamb in their flock. Hence, the high preference of ewes mothering quality might be the indication sheep producers are trying to be profitable by obtaining market demanded and large sized lambs in early ages. In agreement to this, Abebeet al. (2020) indicated that one possible reason of smallholder farmers for selecting of ewes with sound mothering abilities could be selling lambs for income generation, thus well-nourished lambs are expected to fetch a better price. In large scale production system of this study, twining ability was the second most important trait for ranking of breeding ewes. The higher preference for twinning was consistent with the reports of Edea et al. (2012) for Bonga sheep and Nziku et al. (2016) for dairy goats in Kenya.

**Table 2** - Selection criteria for ranking of breeding ewes in smallholder and large-scale system

| Selection Criteria | Smallholder system | | | | Large scale system | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | I | R1 | R2 | R3 | I | I |
| Body conformation | 23.3 | 21.7 | 8.3 | 0.20 | 33.3 | 10.0 | 20.0 | 0.23 | 0.22 |
| Coat colour | 13.3 | 16.1 | 22.2 | 0.16 | 6.7 | 10.0 | 8.3 | 0.08 | 0.12 |
| Mothering ability | 20.6 | 16.1 | 20.6 | 0.19 | 3.3 | 16.7 | 30.0 | 0.12 | 0.16 |
| Age at 1st lambing | 5.0 | 7.2 | 3.9 | 0.06 | 11.7 | 15.0 | 10.0 | 0.13 | 0.09 |
| Twinning ability | 12.2 | 22.2 | 16.1 | 0.16 | 15.0 | 28.3 | 13.3 | 0.19 | 0.17 |
| Lambing interval | 18.9 | 8.9 | 8.3 | 0.13 | 25.0 | 18.3 | 5.0 | 0.19 | 0.16 |
| lamb growth | 6.7 | 7.8 | 20.6 | 0.10 | 5.0 | 1.7 | 13.3 | 0.05 | 0.08 |

R = Rank; I = Index

**Evaluation of farmers ranking decision**

As indicated in table 3, there was a significant difference ($P<0.001$) in the mean values for morph-metric conformation traits across the established ranking groups. Among all ewes and rams studied within the two production systems, those selected as best were highest for all traits than those grouped as poor.

In general, the ewes and rams grouped as average had mean values that were in between the best and poor groups. For instance, in smallholder system, the difference between the best and poor group in live weight for Gumuz sheep was 8.2kg at two years age. Rams ranked as best in both breed groups were higher in all measured traits compared with rams classified as poor qualities (Table 3). For instance, in Gumuz rams the magnitude difference between the best and inferior rams in live weight, body condition and scrotal circumference were 12.7kg, 0.9 and 3.2cm, respectively. In Rutana ram, the differences between the two groups were 13kg of live weight, 1.8 body condition and 1.9 cm scrotal circumferences.

In the present study, there was significant difference between breeds and ranks for all objectively measured traits, which were in accordance with those reported by Gemeda et al. (2010) and Königet al. (2016). Rutana ewes and rams had higher mean values for all traits compared with Gumuz rams and ewes. In addition, in both breeds ewes and rams


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selected as best significantly superior than with that of ranked as poor in all of measured body conformation traits. Inline to this, Sheriff et al. (2021) for Arab and Oromo goat keepers in north western Ethiopia and Getachewet al. (2020) for indigenous goat of the pastoral communities in Ethiopia justified that the mean values of does ranked as best and poor quality, there were clear and logical differences in most of the attributes considered. At the same time, a recent study on Simien sheep breed showed that the best ranked ewes had significantly higher values than the other ranked groups (Solomon et al., 2020). In general, there was a clear trend for the different traits of ewes and rams ranked from best to inferior. Therefore, using of farmers knowledge for selecting the best animals is possible option to start the breeding program where performance recording totally lacking.

**Table 3** - Least squares mean (± SE) of objectively measured traits by breed types and rank categories

| Trait | Breed | Rank | LS (mean ± SE) Ewes | LS (mean ± SE) Rams |
|---|---|---|---|---|
| Body Weight (kg) | Gumuz | Best | 34.5±3.3$^{a}$ | 40.1±6.1$^{a}$ |
| | | Average | 30.2±4.0$^{b}$ | 30.2±4.5$^{b}$ |
| | | Poor | 26.7±4.7$^{c}$ | 27.4±2.6$^{c}$ |
| | Rutana | Best | 39.4±5.7$^{d}$ | 41.4±8.2$^{d}$ |
| | | Average | 35.1±5.0$^{e}$ | 34.5±4.1$^{e}$ |
| | | Poor | 29.5±6.2$^{f}$ | 28.4±5.4$^{f}$ |
| Significance level | *** | *** | | |
| Heart Girth (cm) | Gumuz | Best | 77.1±3.3$^{a}$ | 78.5±4.3$^{a}$ |
| | | Average | 74.2±3.4$^{b}$ | 73.2±3.9$^{b}$ |
| | | Poor | 71.4±3.1$^{c}$ | 73.1±5.1$^{c}$ |
| | Rutana | Best | 82.5±4.1$^{d}$ | 86.4±7.3$^{d}$ |
| | | Average | 79.1±3.9$^{e}$ | 78.7±4.9$^{e}$ |
| | | Poor | 76.2±4.2$^{f}$ | 75.1±5.6$^{f}$ |
| Significance level | *** | *** | | |
| Body Length (cm) | Gumuz | Best | 68.8±2.7$^{a}$ | 69.0±3.9$^{a}$ |
| | | Average | 66.3±2.9$^{b}$ | 65.6±4.0$^{b}$ |
| | | Poor | 63.7±3.1$^{c}$ | 65.4±3.5$^{c}$ |
| | Rutana | Best | 71.1±3.6$^{d}$ | 73.0±5.4$^{d}$ |
| | | Average | 67.7±3.4$^{e}$ | 67.3±4.7$^{e}$ |
| | | Poor | 64.9±3.7$^{f}$ | 64.6±4.3$^{f}$ |
| Significance level | *** | *** | | |
| Wither Height (cm) | Gumuz | Best | 68.9±3.4$^{a}$ | 73.4±8.5$^{a}$ |
| | | Average | 64.7±4.8$^{b}$ | 68.1±6.1$^{b}$ |
| | | Poor | 61.6±5.1$^{c}$ | 69.3±4.0$^{c}$ |
| | Rutana | Best | 77.6±4.8$^{d}$ | 79.5±5.5$^{d}$ |
| | | Average | 73.9±3.8$^{e}$ | 72.7±4.8$^{e}$ |
| | | Poor | 71.6±3.2$^{f}$ | 69.5±5.1$^{cf}$ |
| Significance level | *** | ** | | |
| Body Condition Score | Gumuz | Best | 3.2±0.4$^{a}$ | 3.2±0.8$^{a}$ |
| | | Average | 2.4±0.5$^{b}$ | 2.7±0.5$^{b}$ |
| | | Poor | 1.6±0.4$^{c}$ | 2.3±0.3$^{c}$ |
| | Rutana | Best | 4.0±0.6$^{d}$ | 3.7±0.8$^{d}$ |
| | | Average | 3.0±0.4$^{e}$ | 2.6±0.5$^{e}$ |
| | | Poor | 2.3±0.4$^{f}$ | 1.9±0.6$^{f}$ |
| Significance level | *** | *** | | |
| Scrotal Circumference (cm) | Gumuz | Best | | 26.1±3.4$^{a}$ |
| | | Average | | 22.7±3.1$^{b}$ |
| | | Poor | | 22.9±2.5$^{b}$ |
| | Rutana | Best | | 25.5±3.2$^{d}$ |
| | | Average | | 23.7±3.7$^{e}$ |
| | | Poor | | 23.6±2.6$^{e}$ |
| Significance level | * | * | | |

Significance levels: ***= p <0.001; **= p< 0.01; *= p< 0.05; SE = Standard Error

**Trait preferences of farmers**

Reproduction (lambing interval and twining abilities) and mothering ability were equally the second important traits for appreciated Gumuz ewes in large scale system. The highest weighted reasons reported for Rutana ewes were for appreciating their body size and growth (0.32) in large scale system and, for mothering abilities and body size and growth (equally 0.28) in smallholder system (Table 4). Body condition and reproduction were the second and the third preferred traits in the same breed and production system. Breed behavior was the last preferred trait in both breeds and production systems.



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Reproduction, mothering ability, body size and growth were the most preferred traits for appreciating Gumuz and Rutana ewes in both production systems. This is in agreement with previous studies reported by Solomon et al. (2011) in the same area for the same breed. Inclusion of reproductive traits in designing a breeding program is, however, reasonable as the trait should reflect owners' preferences and will make them more beneficiaries from the sheep production system. Sheep owners' positive view on body size has direct effect in the production of marketable animals with good body conformation, which are later affect to their market price. Higher preference values of body size for breeding animals were reported by many previous studies in Ethiopia (Solomon et al., 2010; Duguma et al., 2011; Solomon et al., 2011). Mothering ability for Rutana sheep in both production systems also considered as the second most important trait in identifying best ewes.

**Table 4** - Smallholder and commercial farmers' ewe trait preference

| Breed and Trait | Smallholder | | | | | | Large scale | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reasons | | | Sum | Rel. wt | Rank | Reasons | | | Sum | Rel.wt | Rank |
| | 1 | 2 | 3 | | | | 1 | 2 | 3 | | | |
| **Gumuz** | | | | | | | | | | | | |
| Body size and growth | 10 | 6 | 4 | 20 | 0.17 | 2 | 4 | 3 | 5 | 12 | 0.20 | 1 |
| Body condition | 8 | 5 | 4 | 17 | 0.14 | 4 | 3 | 3 | 2 | 8 | 0.13 | 4 |
| Mothering abilities | 9 | 5 | 6 | 20 | 0.17 | 2 | 5 | 2 | 2 | 10 | 0.17 | 2 |
| Reproduction | 12 | 8 | 6 | 26 | 0.22 | 1 | 5 | 5 | 0 | 10 | 0.17 | 2 |
| Drought Tolerance | 4 | 4 | 7 | 15 | 0.13 | 5 | 3 | 2 | 3 | 8 | 0.13 | 4 |
| Disease Resistances | 11 | 1 | 6 | 18 | 0.15 | 3 | 2 | 3 | 4 | 9 | 0.15 | 3 |
| Breed behavior | 0 | 1 | 3 | 4 | 0.03 | 6 | 1 | 2 | 0 | 3 | 0.04 | 5 |
| **Rutana** | | | | | | | | | | | | |
| Body size and growth | 14 | 11 | 8 | 33 | 0.28 | 1 | 5 | 7 | 7 | 19 | 0.32 | 1 |
| Body condition | 6 | 3 | 9 | 18 | 0.15 | 2 | 2 | 4 | 1 | 7 | 0.11 | 4 |
| Mothering ability | 8 | 14 | 11 | 33 | 0.28 | 1 | 6 | 4 | 2 | 12 | 0.21 | 2 |
| Reproduction | 8 | 4 | 5 | 17 | 0.14 | 3 | 4 | 2 | 4 | 10 | 0.16 | 3 |
| Drought Tolerance | 1 | 2 | 2 | 5 | 0.05 | 5 | 0 | 1 | 3 | 4 | 0.06 | 6 |
| Disease Resistances | 2 | 2 | 5 | 9 | 0.07 | 4 | 2 | 2 | 1 | 5 | 0.09 | 5 |
| Breed behavior | 2 | 2 | 0 | 4 | 0.03 | 6 | 1 | 1 | 1 | 3 | 0.05 | 7 |

Reasons 1,2,3= Farmers 1st, 2nd and 3rd basis for evaluating ewes.

## CONCLUSION

Smallholder farmers primarily selected body conformation with ability to give multiple births and decent mothering ability whereas; multiple births and lambing interval were important selection criteria by large scale producers for breeding ewes. Physical appearance traits like body conformation and growth rate were principally considered for selection of breeding rams in both production systems. In general, traits, which have direct influence on the market price,are highly marked as a selection criterion for breeding rams and ewes. The top three preferred traits according to the weighted rank values were reproduction, body size and mothering ability. The main breeding goals for both systems have been defined as increasing meat production and marketed animals. This implies that designing sheep improvement strategy in the area should primarily target towards meat production traits.

## DECLARATIONS

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**Authors' contribution**
All of authors contribute on idea conception, data collection and analysis, and the write up of the manuscript.

**Conflict of interests**
The authors have not declared any conflict of interests.

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# EFFECT OF COCONUT MILK INCLUSION IN ROOT MEAL-BASED DIETS ON PERFORMANCE AND FEED INTAKE OF NATIVE CHICKENS

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**ABSTRACT**: In pursuit of feed cost reduction for chickens, a reduced feed intake strategy was proposed, but how this strategy works without compromising the chicken performance rarely explored. This study proposes the satiating effects of fresh coconut milk (FCM) as dietary fat for giant swamp taro meal-based (GST) diets on the performance and feed intake reduction of native chickens. One hundred eighty Bisaya native chickens have been grouped into FCM-free and FCM supplemented groups further divided into 0%, 25%, and 50% GST sub-groups of ten chickens in each sub-group. The experiment was laid out in a 2 × 3 factorial in a completely randomized design. The feeding trial started on week 5 post-hatch and terminated on week 12 post-hatch. The average daily feed intake (ADFI) and weight gain (WG) of chicken at weekly intervals were highly significant among FCM as well as GST treatments during weeks 5–7, also the final ADFI and WG. However, no remarkable differences of ADFI and WG in weeks 8–12 feeding. The bodyweight of chickens was highly significant among the FCM group and levels of GST were consistently observed throughout the study period. Neither the FCM nor GST groups differ the values for FCR. However, a bit higher FCR for FCM supplemented and 0% GST treatments. Slaughter weight, meat cuts (breast and thigh) and organ weight (liver, gizzard, and heart) was uninfluenced by either FCM or levels of GST. FCM supplemented diet had higher apparent digestibility of crude ash than FCM-free. 25% GST meal inclusion had higher apparent digestibility of crude fiber compared to 0% and 50% GST. No remarkable FCM×GST interactions in all parameters tested throughout the trial. In conclusion, supplementation of fresh coconut milk to giant swamp taro meal as replacement to maize did not reduce feed intake of chickens but rather increased body weight, weight gain with marginal improvement in FCR. The 50% giant swamp taro replacement to maize compromises overall performance of native chickens. However, supplementing fresh coconut milk to giant swamp taro meal can replace maize up to 25% that gives a better body weight, weight gain, and feed conversion ratio.

**Keywords:** Bisaya native chicken, Coconut, Dietary fats, Feed efficiency, Root crops.

## INTRODUCTION

Feed cost accounts for 70–80% of the total production expenses for poultry farms (Mallick et al., 2020), but what many feed millers and researchers may not realize is how to develop a diet that has higher satiation effects that will reduce feed intake to help alleviate the current problem. Satiation means the process which brings eating to a halt, while satiety is the state of inhibition over further eating (Blundell, 1984). Satiation occurs during an eating episode and brings it to an end. Satiety starts after the end of eating and prevents further eating before the return of hunger. Enhancing satiation and satiety derived from foodstuffs was perceived as a means to facilitate feed intake and weight control (Bellisle et al., 2012).

Hunger and satiety are affected by the nutritional composition and structure of foods: therefore, some foods have a greater capacity to maintain suppression over appetite than others (Pickering and Halford, 2016). When expressed relative to energy content rather than the weight of food, protein exerts the most substantial effect on satiety, followed by carbohydrate, while fat exerts the weakest effect (Blundell et al., 1993; Hopkins et al., 2016). In humans, the protein content of a food or meal is also a factor in the short-term reduction of food intake (Anderson and Moore, 2004). Because protein as a feed ingredient for chicken is scarcer than carbohydrates, providing a high-protein diet beyond tissue building is inefficient. The study between low-fat high carbohydrate (LFHC) and high fat low carbohydrate (HFLC) for overweight and obese individuals suggests that LFHC foods promote reduced energy intake (Hopkins et al., 2016), demonstrating LFHC diets are effective for long-term weight loss. However, high carbohydrate as energy feed ingredients is the largest in terms of quantity (40-70%) for a poultry diet and are becoming scarce and invariably the most expensive (Skinner et al., 1992; Van der Klis, 2010) due to stiff competition as it used by industries for biofuel and as food for humans (Ahiwe et al., 2018). Fats are the most energy-dense of the macronutrients at 9.5 kcal per gram, and high-fat diets (in comparison with low-fat and high-carbohydrate diets) have a disproportionately weak action on satiety (Pickering and Halford, 2016). In terms of affordability and availability, fats can be considered the cheapest and the most abundant than other macronutrient proteins and carbohydrates (Ravindran et al., 2016).



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The use of non-conventional proteins and energy sources to substitute soybean and maize in monogastric animals is currently a worldwide effort. Some non-conventional feed ingredients such as the "Palau" root crop (*Cyrtosperma merkusii*) are commonly used to replace maize due to their availability, being less utilized by humans, and being of economic importance (Taer and Taer, 2020). However, taro diet inclusion is very poor due to anti-nutritional factors that decreased feed intake and growth performance. Additionally, the problem with this root crop was that taro has lower nutrient densities affecting performance and efficiency than maize and other non-conventional feedstuffs (Temesgen and Retta, 2015; Temesgen et al., 2017).

The addition of fresh coconut milk as dietary fat for a root meal-based diet will improve the energy density of root starches and is expected to enhance the productivity of poultry. The effect of fats on satiety has been investigated in four areas associated with fat structure: chain length, degree of saturation, degree of esterification, and functionality of specific fat molecules (Samra, 2010). In fat chain length, coconut milk has a high percentage of a medium-chain fatty acid beneficial to increase weight without increasing cholesterol levels (St-Onge and Jones, 2002; Wallace, 2019). Coconut consists of fatty acids that work as the source of energy and antimicrobial effects. The study of coconut milk as one alternative to improve the immune system when birds are in stress or uncomfortable housing conditions (Shakeri et al., 2016). Dietary fat addition slows down digestion passage rate through the gastrointestinal tract, allowing better nutrient digestion, absorption, and utilization (Mateos et al. 1982; Latshaw, 2008), probably through increased contact with digestive enzymes. Several mechanisms, including regulation of Ghrelin (appetite hormones) and inhibition of gastric emptying and intestinal transit, are perceived fats that affected satiety (Samra, 2010). A study in humans also found that food intake at lunch was lower after a high medium chain triglyceride (MCT) breakfast when compared to high oleic or high saturated fat breakfast in men (Van Citters and Lin, 1999). Another research found lower intake at dinner after a high medium chain triglycerides consumption at lunch (Van Wymelbeke et al., 1998). The reason for using coconut milk instead of coconut oil was related to high levels of saturated fat in coconut oil which can increase the level of harmful LDL-cholesterol in the blood (Gómez et al., 2000).

While these findings indicate that the higher inclusion of medium-chain fatty acids in coconut milk potentially lowers feed intake and increased weight without increasing cholesterol level in humans, the effect of the same, on feed intake and weight gain for chickens has yet to be determined. Hence, this study aimed to investigate the effects levels of giant swamp taro meal as a replacement for maize added with or without coconut milk on the growth performance, feed intake reduction, and efficiency of native chicken. This preliminary study focused on the performance and feed intake reduction effect and fat accumulation of fresh coconut milk as dietary fat concerning satiety.

## MATERIALS AND METHODS

### Study location and ethical regulations

The study was supervised and approved by the research committee of the department of agriculture, in compliance with the rules and regulations on the scientific procedures using animals under the Philippines Republic No. 8485, otherwise known as the "Animal Welfare Act of 1998". The experimental setup was at the poultry facility in the Department of Animal Science of Surigao State College of Technology-Mainit Campus, in Mainit Surigao del Norte, Philippines on January to April, 2021.

### Research design and treatment

This experimental study was a two-factor factor experiment arranged in a Complete Randomized Design (CRD). The first factor was FCM-free and FCM supplemented while the second factor was 0%, 25%, and 50% levels of giant swamp taro meal-based (GST) composed of 6 treatments replicated 3 times. Each replication consisted of 10 heads of 5-weeks post hatch "Bisaya" native chickens having 282.00 ± 2.00 g average initial weight. The study was started at 5-weeks post hatched and terminated at 12-weeks post hatched. Treatment combinations were the following: T1 = FCM-supplemented-0% GST; T2 = FCM-supplemented-25% GST; T3 = FCM-supplemented-50% GST; T4 = FCM-free-0% GST; T5 = FCM-free-25% GST; T6 = FCM-free-50% GST.

### Preparation of giant swamp taro meal

Giant swamp taro corms were taken from Pongtud, Alegria, Surigao del Norte and adjacent barangay of Magpayang, Mainit, Surigao del Norte where SSCT-Mainit Campus experimental station was located. Preparation for Giant swamp taro corm's was in accordance by procedures of Taer and Taer (2020).

### Extraction of fresh coconut milk

Mature coconuts were obtained from a farmer. Nuts were cleaned de-husked, break it open, drained the coconut water, and then mechanically grated. Every 10 kg of grated coconut meat was mixed with 1 liter distilled water. The mixture of coconut meat and distilled water were blended in high-speed blender for 60 seconds and poured the contents in a pan with thin muslin. The coco milk was strained, filtrate was squeezed by hand, consequently collected the fresh coconut milk in a clear bottle and store in a refrigerator until use.

### Experimental diet formulation and mixing of fresh coconut milk

The experimental diets were formulated in which the chicken grower and finisher diets were calculated to contain 3000 ME kcal/kg and 22% CP and 2800 ME kcal/kg and 20% crude protein respectively. Feed ingredients (micro and


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macro) were prepared and weighed separately according to formulation using a digital weighing scale (Table 1). Micro-ingredients were the first to mix by hand before they were incorporated with the macro-ingredients using the mechanical mixer. The mixed feeds were partitioned into two fractions. The first fraction was allocated as GST treatment without fresh coconut milk whereas the second fraction was mixed with fresh coconut milk (FCM) at a ratio of 100ml fresh coconut milk in every kilogram of feeds. The mixing of coconut milk with the diets was done on a daily basis to avoid feed spoilage due to coconut milk addition.

### Data collection

Initial weight of birds was gathered upon start of feeding trial (week - 5 post-hatch) and repeated weekly thereafter to get the weekly weight increment and weight gain. The total feed given and total feed refused were weighed and recorded daily and some birds that died during the experiment in each replicate. Feed conversion ratio (FCR) was calculated as grams of feed consumed per grams weight gain in birds in each pen. At the end of the experiment (week - 12 post-hatch), all the birds were fasted overnight, stunned electrically and slaughtered by decapitation. Slaughtered birds were scalded at 50 $^0$C for about 1 min, plucked manually, eviscerated and dressed. The carcass cuts (breast meat and thigh) organ (liver, gizzard, heart) were expressed as percentages (g/kg) of the live weight. On the seventh week of feeding, the daily excreta voided per cage were collected via plain G.I. sheet measuring 30 × 60 cm used to catch the excreta under the cage. To avoid contamination with feeds and other contaminants, the excreta were collected every 4 hours for the period of seven days. The excreta were oven-dried at 60$^0$C for 12 hours and kept frozen (-200C) until it's ready for analysis.

### Statistical analysis

Data collected were subjected to ANOVA of the GLM in SPSS (SPSS for Windows, version 26.0; IBM Corp., Armonk, NY, USA), using the pen as the experimental unit for live weight, weight gain, average daily feed intake, and FCR. Slaughter weight, carcass and organ weights measured on individual birds and related to pen as the experimental unit. Treatment means were compared using the Tukey's honestly significant difference (HSD) test and differences were considered significant at 5% level of probability.

**Table 1 -** Composition of grower and finisher diet in the experiment (as fed-basis) Giant swamp taro root meal

| Ingredients | Starter Mash | | | Finisher Mash | | |
|---|---|---|---|---|---|---|
| | Corn-based | 25% GST | 50% GST | Corn-based | 25% GST | 50% GST |
| Yellow corn | 56.00 | 42.00 | 28.00 | 48 | 36.00 | 24.00 |
| GST | - | 14.00 | 28.00 | - | 12.00 | 24.00 |
| Rice Bran D1 | 7.00 | 7.00 | 7.00 | 15.00 | 15.00 | 15.00 |
| Soybean Meal | 16.00 | 16.00 | 16.00 | 15.00 | 15.00 | 15.00 |
| Fish meal | 14.00 | 14.00 | 14.00 | 7.00 | 7.00 | 7.00 |
| Copra meal | 4.00 | 4.00 | 4.00 | 13.00 | 13.00 | 13.00 |
| Coco oil | 1.00 | 1.00 | 1.00 | 0.50 | 0.50 | 0.50 |
| Molasses | 1.00 | 1.00 | 1.00 | 0.50 | 0.50 | 0.50 |
| Limestone | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |

## RESULTS

### Average daily feed intake

Average daily feed intake (ADFI) of chickens during weeks 5–7 ranges 20.40–48.64g higher for FCM supplement, while 18.24g–47.63g lower for FCM-free ($p<0.01$) chickens (Table 2). The 21.27–49.18g highly significantly ($p<0.01$) different to 50% GST had 16.65-46.10g, however, 25% GST had 20.04–49.01g did not differ to 0% GST during the same period. No ADFI differential in the FCM and GST treatments during 8-weeks grower feeding to the rest of finisher period as well no FCM*GST interactions in ADFI in the entire experiment.

### Live weight

As shown in Table 3, the differences initial body weight was found to be non-significant ($p>0.05$) whereas live weight at 5-weeks and onwards were highly significant ($p<0.01$). The significant to highly significant differences of live weights were constantly observed in both FCM and GST level groups. Birds with FCM supplement had higher ($p<0.01$) body weight compared with FCM-free. Birds under 0% GST were also higher ($p<0.01$) in body weight from 5 weeks – 12 weeks compared to birds under 50% GST. However, the values for 25% GST birds were not significantly ($p>0.05$) different to 0% GST. No significant FCM*GST interactions noted in weekly body weights of native chickens.



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### Weight gain

Weekly weight gain of chickens from weeks 5–7 pot-hatch shows a highly significant ($p<0.01$) differences between FCM supplement and FCM-free as well as in between GST levels as presented in Table 4. Increased weight gain in FCM supplement ranges 7.91–18.75% higher ($p<0.01$) versus the FCM-free (54.34g vs. 44.15g, 70.45g vs. 59.74g, and 99.41g vs. 92.26g), respectively. The counterpart 0% GST was also 13.42 – 35.80% higher ($p<0.01$) than 50% GST within these periods. Although, the same treatment was 8.34–10.97% higher over the 25% GST but the differences were unremarkable. No remarkable weight gain differential in chickens in FCM and GST treatments during finisher phase feeding.

### Feed conversion ratio

Figure 1 shows the cumulative feed conversion ratio (FCR) on native chicken fed with FCM-free and FCM supplement with levels of GST. The results showed no significant difference ($p>0.05$) FCR values. However, an improved FCR recorded for FCM supplement (3.14) than FCM-free (3.22). The FCR for 0% GST (3.11) was better than the FCR for 25% GST (3.19) and 50% GST (3.26).

### Carcass weight, weight meat cuts and organ weight

The summary weight of carcass component, meat cuts and weight of internal organs of native chicken are shown in Table 5. All attributes viz. dress weight, dressing percentage, breast weight, thigh weight, liver, gizzard and heart weight are not significant ($p>0.05$) in all treatments except for abdominal fat. The FCM was 1334.27g higher than no FCM 1284.25g while the 0% GST was 1352.00g followed by 50% GST 1299.64g then 25% GST 1276.85g but their value differences were not significant.

### Apparent nutrient digestibility

Apparent nutrient digestibility of crude protein, crude ash, crude fiber, and nitrogen-free extract (NFE) were tested in this study and are presented in Table 6. The ANOVA for apparent digestibility of crude fiber showed a significant ($p<0.05$) difference within levels of GST treatments. Apparent digestibility in crude ash revealed a significant higher digestibility in FCM treatments. The rest of the nutrients viz. apparent digestibility of crude protein and NFE showed no remarkable differences ($p>0.05$) in all treatments.

**Table 2** - Average daily feed intake of native chicken fed FCM-free and FCM supplement at different levels of GST

| Items | | Grower Phase (g) | | | | Finisher Phase (g) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Week 5 | Week 6 | Week 7 | Week 8 | Week 9 | Week 10 | Week 11 | Week 12 |
| FCM | With FCM | 20.40$^{a}$ | 32.58$^{a}$ | 48.64$^{a}$ | 59.52 | 70.24 | 79.26 | 89.97 | 105.92 |
| | FCM-free | 18.24$^{b}$ | 29.34$^{b}$ | 47.63$^{b}$ | 60.04 | 69.14 | 78.69 | 89.56 | 107.00 |
| GST | 0% | 21.27$^{a}$ | 34.07$^{a}$ | 49.28$^{a}$ | 59.79 | 70.50 | 78.41 | 90.42 | 106.40 |
| | 25% | 20.04$^{a}$ | 32.39$^{a}$ | 49.01$^{a}$ | 60.21 | 69.32 | 79.93 | 89.12 | 106.90 |
| | 50% | 16.65$^{b}$ | 26.43$^{b}$ | 46.10$^{b}$ | 59.34 | 69.25 | 78.59 | 89.77 | 106.08 |
| *p*-Value | FCM | 0.00777 | 0.03122 | 0.04022 | 0.43505 | 0.02468 | 0.51169 | 0.57538 | 0.29596 |
| | GST | 0.00036 | 0.00024 | 0.00011 | 0.56414 | 0.06181 | 0.31701 | 0.36477 | 0.79304 |
| | FCM*GST | 0.2111 | 0.16431 | 0.13897 | 0.1408 | 0.13972 | 0.75981 | 0.28826 | 0.34422 |

Column means of the same letter are not significantly different at 0.05 level; GST= giant swamp taro; FCM = fresh coconut milk.

**Table 3** - Weekly live weight of native chickens fed FCM-free and FCM supplement at different levels of GST

| Items | | Grower Phase (g) | | | | Finisher Phase (g) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Week 5 | Week 6 | Week 7 | Week 8 | Week 9 | Week 10 | Week 11 | Week 12 |
| FCM | With FCM | 282.07 | 336.41$^{a}$ | 406.90$^{a}$ | 506.33$^{a}$ | 627.99$^{a}$ | 777.92$^{a}$ | 968.64$^{a}$ | 1175.16$^{a}$ |
| | FCM-free | 282.15 | 326.31$^{b}$ | 386.06$^{b}$ | 478.33$^{b}$ | 596.49$^{b}$ | 739.11$^{b}$ | 925.88$^{b}$ | 1133.78$^{b}$ |
| GST | 0% | 282.16 | 340.52$^{a}$ | 415.37$^{a}$ | 516.18$^{a}$ | 645.48$^{a}$ | 795.00$^{a}$ | 985.52$^{a}$ | 1196.72$^{a}$ |
| | 25% | 282.16 | 334.11$^{a}$ | 400.91$^{a}$ | 498.20$^{a}$ | 616.70$^{b}$ | 763.42$^{a}$ | 957.23$^{a}$ | 1164.92$^{a}$ |
| | 50% | 282.01 | 319.46$^{b}$ | 373.17$^{b}$ | 462.60$^{b}$ | 574.54$^{c}$ | 717.12$^{b}$ | 899.03$^{b}$ | 1101.78$^{b}$ |
| *p*-Value | FCM | 0.746 | 0.00181 | 0.00124 | 0.00034 | 0.00129 | 0.00435 | 0.00654 | 0.02662 |
| | GST | 0.854 | 0.00006 | 0.00005 | 0.00002 | 0.00002 | 0.00034 | 0.0005 | 0.00157 |
| | FCM*GST | 0.574 | 0.64075 | 0.21454 | 0.29834 | 0.35775 | 0.30835 | 0.17798 | 0.15411 |

Column means of the same letter are not significantly different at 0.05 level; GST= giant swamp taro; FCM = fresh coconut milk.



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**Table 4 -** Weight gain of native chicken fed FCM-free and FCM supplement at different levels of GST

| Items | | Grower Phase (g) | | | | Finisher Phase (g) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Week 5 | Week 6 | Week 7 | Week 8 | Week 9 | Week 10 | Week 11 | Week 12 |
| FCM | With FCM | 54.34[a] | 70.48[a] | 99.41[a] | 121.65 | 149.93 | 190.70 | 206.52 | 235.21 |
| | FCM-free | 44.15[b] | 59.74[b] | 92.26[b] | 118.16 | 142.61 | 186.77 | 207.90 | 233.59 |
| GST | 0% | 58.34[a] | 74.84[a] | 100.80[a] | 129.28[a] | 149.52 | 190.51 | 211.21 | 235.82 |
| | 25% | 51.94[a] | 66.79[a] | 97.29[a] | 118.49[ab] | 146.71 | 193.81 | 207.69 | 231.18 |
| | 50% | 37.45[b] | 53.70[b] | 89.43[b] | 111.93[b] | 142.59 | 181.90 | 202.74 | 236.20 |
| *p*-Value | FCM | 0.00149 | 0.01634 | 0.00461 | 0.41022 | 0.18764 | 0.41319 | 0.78146 | 0.76263 |
| | GST | 0.00006 | 0.00252 | 0.00218 | 0.01482 | 0.5694 | 0.13816 | 0.38607 | 0.69369 |
| | FCM*GST | 0.66868 | 0.19358 | 0.343 | 0.81042 | 0.45699 | 0.21884 | 0.16057 | 0.74115 |

Column means of the same letter are not significantly different at 0.05 level; GST= giant swamp taro; FCM = fresh coconut milk.

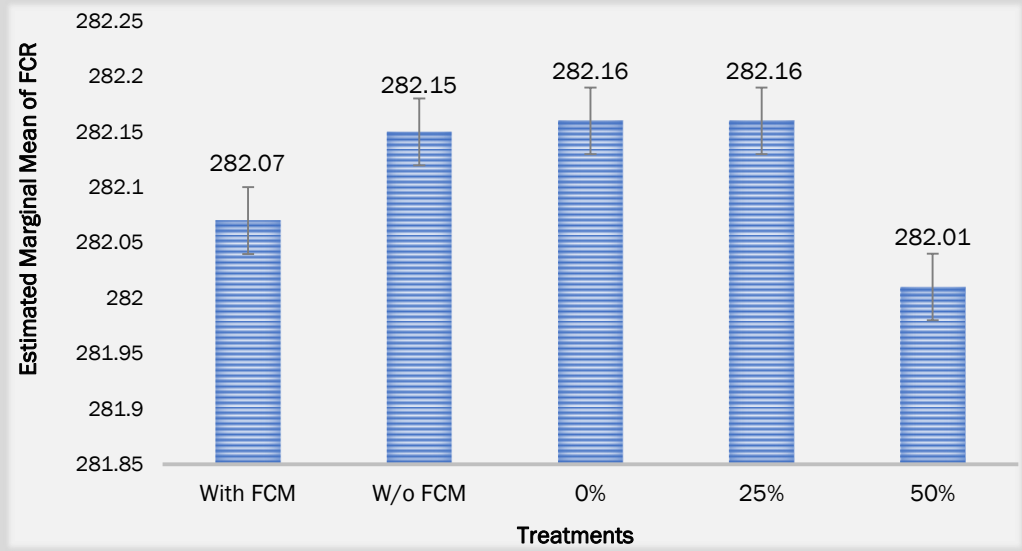


**Figure 1 -** Feed conversion ratio of native chicken fed FCM-free and FCM supplement at different levels of GST.

**Table 5 -** Carcass quality, meat cuts, and organ weights of native chicken fed FCM-free and FCM supplement at different levels of GST

| Item | | Slaughter weight (g) | Carcass Harvest | | Meat Cuts* | | Internal Organs* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Dress weight (g) | Dressing Percentage (%) | Breast (%) | Thigh (%) | Liver (%) | Gizzard (%) | Heart (%) |
| FCM | With FCM | 1334.77[a] | 901.76 | 67.76 | 19.98 | 18.70 | 3.46 | 4.69 | 1.48 |
| | FCM-free | 1284.25[b] | 921.21 | 72.00 | 20.99 | 19.08 | 3.56 | 4.04 | 1.32 |
| GST | 0% | 1352.00[a] | 911.52 | 67.57 | 20.11 | 17.10 | 3.71 | 4.60 | 1.16 |
| | 25% | 1276.85[b] | 924.29 | 72.58 | 22.00 | 20.13 | 2.93 | 4.04 | 1.72 |
| | 50% | 1299.64[ab] | 898.63 | 69.49 | 19.29 | 19.53 | 3.90 | 4.46 | 1.33 |
| *p* Value | FCM | 0.01567 | 0.39346 | 0.09966 | 0.88407 | 0.73754 | 0.75311 | 0.20942 | 0.40259 |
| | GST | 0.01455 | 0.6452 | 0.25994 | 0.41552 | 0.26384 | 0.05811 | 0.63211 | 0.08672 |
| | FCM*GST | 0.46286 | 0.24951 | 0.24915 | 0.89859 | 0.79448 | 0.30431 | 0.53649 | 0.32673 |

Column means of the same letter are not significantly different at 0.05 level; *g/kg slaughter weight; GST= giant swamp taro; FCM = fresh coconut milk.



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**Table 6 -** Apparent digestibility of nutrient of chickens fed FCM-free and FCM supplement at different levels of GST.

| Item | Apparent digestibility of nutrient | Crude protein | Crude Ash | Crude fiber | NFE |
|---|---|---|---|---|---|
| FCM | With FCM | 54.62 | 76.16[b] | 66.51 | 55.71 |
| | FCM-free | 54.27 | 79.41[a] | 65.91 | 55.93 |
| GST | 0% | 53.71 | 78.86 | 68.08[a] | 55.24 |
| | 25% | 54.85 | 76.97 | 64.91[b] | 56.11 |
| | 50% | 54.77 | 77.54 | 65.63[ab] | 56.10 |
| *p* Value | FCM | 0.52168 | 0.02667 | 0.54434 | 0.80387 |
| | GST | 0.19252 | 0.49243 | 0.04514 | 0.66232 |
| | FCM*GST | 0.82205 | 0.07812 | 0.46973 | 0.16685 |

Column means of the same letter are not significantly different at 0.05 level; GST= giant swamp taro; FCM = fresh coconut milk.

**Table 7 -** Mean and standard deviations of treatment combinations on overall growth performance (body weight, weight, ADFI and FCR) of chickens fed FCM-free and FCM supplement at different levels of GST

| Treatment Combinations | Growth Performance | | | |
|---|---|---|---|---|
| | Body Weight (Final) | Weight Gain (Final) | ADFI (Final) | FCR |
| 0% GST-FCM | 1435.68 ± 51.94 | 1153.43 ± 11.76 | 63.94 ± 0.43 | 3.11 ± 0.12 |
| 25% GST-FCM | 1439.94 ± 9.98 | 1157.74 ± 8.19 | 63.85 ± 0.69 | 3.09 ± 0.03 |
| 50% GST-FCM | 1355.53 ± 46.41 | 1073.76 ± 4.88 | 62.16 ± 0.74 | 3.25 ± 0.10 |
| 0% GST- FCM-free | 1429.43 ± 15.10 | 1147.33 ± 12.28 | 63.60 ± 0.44 | 3.10 ± 0.53 |
| 25% GST- FCM-free | 1352.28 ± 51.79 | 1070.15 ± 17.01 | 62.88 ± 0.45 | 3.29 ± 0.14 |
| 50% GST- FCM-free | 1320.44 ± 41.36 | 1038.19 ± 8.74 | 60.89 ± 0.90 | 3.29 ± 0.08 |

ADFI= average daily feed intake; FCR= feed conversion ratio; GST= giant swamp taro; FCM = fresh coconut milk.

**Table 8 -** Correlation analysis of live weight (final), weight gain (final), ADFI (final) and FCR of chicken

| | Live weight (Final) | Weight gain(Final) | ADFI (Final) | FCR |
|---|---|---|---|---|
| **Live weight (Final)** | 1 | | | |
| **Weight gain (Final)** | 1.000** | 1 | | |
| **ADFI (Final)** | 0.853** | 0.854** | 1 | |
| **FCR** | -0.959** | -0.958** | -0.672* | 1 |

*= Correlation is significant at the 0.05 level; **= Correlation is significant at the 0.01 level; ADFI= average daily feed intake; FCR= feed conversion ratio;

## DISCUSSION

The aim of this study was to evaluate the effect of levels of giant swamp taro root meal as substitute to corn with and without fresh coconut milk supplementation on performance indices and differentiate its rule in feed intake and efficiency of native chicken. In this study, a decreased body weight and weight gain were consistently detected for 50% GST regardless of FCM supplementation in the entire duration of the experiment. Overall, the final body weight (Table 7) decreased in the 50% GST among levels GST treated group chickens which were inconsistent with Abdulrashid and Agwunobi (2009) using the same GST concentration or with Caicedo et al. (2018) using 40% GST concentrations were reported no effect on productivity of broiler chickens and growing pigs, respectively. Interestingly, from week 1 – week 8 in this experiment, live weight (LW) of chickens did not differ significantly among 0% GST and 25% GST treatments. The highly significant differential in LW of 0% and 25% GST over the 50% GST indicates feed stress due to higher concentrations of anti-nutritional factors (ANF) of the diet. The higher taro inclusion, the higher concentrations of anti-nutritional substances in the diet. Temesgen and Retta (2015) reported that anti-nutritional elements commonly observed in all species of the Araceae family are abundant in most parts of the plant, causing throat irritation and mouth epithelium and indirectly reducing the digestibility. In FCM group, a more pronounced body weight recorded in FCM supplemented birds. The highly significant body weight of chickens under FCM treatment versus no FCM indicates



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supplementation effect of fresh coconut milk. Coconut milk consists high percentage of medium-chain fatty acid which is useful to increase weight without increasing cholesterol levels (St-Onge and Jones, 2002), rich in protein (Capulso et al., 1981) which can provide more essential amino acids for the body (Mepba and Achinewhu, 2003).

Average daily feed intake (ADFI) at weekly interval shared a similar weekly pattern with weight gain. The result indicates a positive correlation between ADFI with weight gain. Correlation analysis (Table 8) showed a highly significant positive correlation between weight gain and ADFI (0.854) whereas a perfectly positive correlation between weight gain and LW (1.000**). This shows that increase in feed intake would lead to a higher LW and weight gain. However, correlation between weight gain, live weight (LW) and ADFI with the FCR was a very highly significant negative correlation (-0.958). Similar to BW outcome, the most improved weight gain was with 0% GST and 25% GST treated birds than did the counterpart 50% GST treatment. The result is expected as the same treatments recorded the highest average final ADFI from weeks 1-8 at 63.76g and 63.36g, respectively (Table 7) and the most efficient feed converter at 3.11 and 3.19 (Figure 1). The reduced ADFI for 50% GST during first 3 week of experiment indicates that younger chickens do not accept the diet probably because of ANF. A major problem affecting the utilization of GST as feed ingredient has been its irritation (Pham et al., 2005), mainly due to the physical structure of oxalic acid needles that reduces palatability. The similarity in ADFI among GST treatments during 7–12 weeks in the present study suggests no palatability problem of the diets for older chickens. The improved final weight gain of chickens in FCM supplemented chickens over the no FCM can be speculated to FCM supplementation. The study of coconut milk diet supplementation to broilers exposed to environmental stress of high stocking densities found an improved immune system even when chickens were subjected to external stressors of high stocking density (Shakeri et al., 2016). In this study, feed stress to birds were apparently observed in no FCM supplementation during early weeks of feeding (5–7-weeks), whereas the higher ADFI on birds supplemented with FCM indicates no palatability problem despite ANF which probably mitigated by the FCM. However, as the birds grew older (8-12 weeks) the FCM effects were unobserved showing that older birds may have adopted the diets.

Dietary fat addition has been found to slow down digesta passage rate through the gastro-intestinal tract, allowing better nutrient digestion, absorption and utilization (Mateos et al. 1982; Latshaw, 2008), probably through increased contact with digestive enzymes. This could be a possible explanation for the improved performance of birds fed FCM despite the similarities in ADFI during last 5 weeks (8-weeks – 12-weeks) of experimentation. Dietary unsaturated fat has also been reported to increase protein accretion in broilers (Sanz et al., 2000), probably by sparing protein from being used as energy. Firman et al. (2010) observed that fat addition decreased FI and improved feed efficiency in broilers. This observation was nullified in this present study, FI was not reduced by FCM addition but rather improved the body weight, weight gain with marginal improvement in FCR. The similarities in FCR findings within FCM and GST groups was attributed to a similarly higher digestibility of crude protein as reflected in Table 6. Correlation analysis for FCR and digestibility of crude protein showed a low positive correlation of 0.216 correlation size (Figure not shown). Adequate consumption of dietary protein is critical for the maintenance of optimal health during normal growth and aging (Carbone and Pasiakos, 2019). In general, the highlight of this study was that FCM treatment performs better than no FCM supplement wherein the 25% GST plus FCM had higher final body weight and weight gain compared to the rest of treatment combinations.

All attributes, viz. dress weight, and dressing percentage, weight of meat cuts (breast and thigh) weight of internal organs (heart, gizzard, and liver) was not influenced ($P>0.05$) by the tested diets. However, the slaughter weight of chickens in this study ranges from 1284.25 – 1334.77g between FCM group and 1276.85 – 1352.00g among GST group was behind the average normal slaughter weight range of 1385 – 1512g for Chee Crossbred Native Chicken raised in 12 weeks (Promket et al., 2016), 1353.13 – 1717.94g for different Aseel crosses raised for 12 weeks (Ullengala et al., 2020). The diet composition and genotype used by the works of Promket et al. (2016) and Ullengala et al. (2020) likely contributed the depressed slaughter weight observed in this present trial.

The dressing percentage was calculated as the ratio between warm carcass weight and live weight at slaughter time. The comparable dress weight and dressing percentage between GST and FCM groups for this trial aligned with dress weight of broilers fed 100% raw and boiled taro (Abdulrashid and Agwunobi, 2009) and dressing percentage of broilers fed 3-12% sun-dried taro corm's meal (Getiso et al., 2021). The result indicates no effect of feed stress on meat harvest. However, these values were lower in dress weight and dressing percentage for Aseel crosses ranges 932.13 – 1194.25g and 68.49 – 69.51%, respectively (Ullengala et al., 2020).

Breast and thigh weight across different treatments were not influenced by FCM and GST addition. These results confirmed no significant changes in breast and thigh weight at 0, 25, 75, and 100% raw and boiled taro inclusion reported by Abdulrashid and Agwunobi, (2009) while inconsistent with Getiso et al. (2021) who found significant breast and thigh among control and four (3, 6, 9, 12%) levels of sundried taro inclusion. However, the 18.92 - 23.24% range of breast (%) and 17.33 - 19.98% thigh (%) were higher than 14.68 – 15.82% breast (%) for different Aseel crosses (Ullengala et al., 2020) and 12.96 – 14.10% thigh (%) for Chee crossbred native chicken (Promket et al., 2016). Weight of internal organs viz. liver, gizzard and heart in this study ranges 2.93 – 4.23, 3.79 – 4.58, and 1.19 – 1.66, respectively. These value ranges however, was higher over Shakeri et al. (2016) having 1.62 – 1.87% for liver, 3.40 – 3.75% for gizzard, and 0.41 – 0.49 for heart. The reasons for the discrepancies on carcass harvest, weight of meat cuts and weight of organs were speculative but may be due to the different levels of added GST in the diet, age at which GST is added to diet, species, sex and native chicken genotype, chick weight at which GST was started to introduced or basal diet


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composition.

Apparent digestibility of nutrients such as crude protein, crude ash, crude fiber and nitrogen-free extract (NFE) in this study were determined. The result showed no remarkable differences in all treatments except crude fiber. The non-significant variations in the apparent digestible crude protein among the treatment diet may be due to high quality of the diet as apparent crude protein digestibility has been reported to depend on the source and concentration of the protein in the feed stuff (McDonald et al., 1991). This finding supports the reports of Ajetunmobi et al. (2020) on non-significant variation in the digestibility of CP, but nullifies the reports of Oso et al. (2014) and Aguihe et al. (2015) on significant variation in the digestible crude protein of broiler chickens fed cassava root meal supplemented with or without charcoal and fed cassava peel meal-based diet with enzyme Maxigrain® supplementation. The significant digestibility of crude ash in this study was higher for birds without FCM (79.41) than the FCM added diets 76.16). whereas, the non-significant results for nitrogen-free extract (NFE) nullifies the report of Ajetunmobi et al. (2020) who found a significant digestibility of NFE on birds fed varying levels of processed taro cocoyam (*Colocasia esculenta*) meal-based diet. Moreover, the significant findings of apparent digestibility of crude fiber among GST groups disagrees with the reports of non-significant digestibility of crude fiber for chickens fed varying levels of taro cocoyam (Ajetunmobi et al., 2020).

## CONCLUSION

Supplementation of fresh coconut milk to giant swamp taro meal as replacement to maize do not reduce feed intake of chickens but rather increased body weight, weight gain with marginal improvement in FCR. The 50% giant swamp taro replacement to maize compromises overall performance of native chickens. However, supplementing fresh coconut milk to giant swamp taro meal can replace maize up to 25% that gives a better body weight, weight gain, and feed conversion ratio.

## DECLARATIONS

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**Authors' contribution**
Taer A, Taer E, contribute to the research and manuscript writing. Escobal E, Alsong L, and Maglinte R. contributed the concept, diet analysis and formulation, statistical analysis and logistics.

**Conflict of interests**
The authors have not declared any conflict of interests.

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# TURKEY MEAT HYGIENE AND BIOLOGICAL SAFETY ASSESSMENT AFTER DEFROSTING

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*Supporting Information*

RESEARCH ARTICLE

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**ABSTRACT**: Violations of temperature and humidity storage conditions and the intensive development of lactic acid putrefactive microorganisms (micrococcus, yeast, and mold fungi) lead to destructive changes in muscle tissue. The studies established the effect of single and repeated defrosting of turkey meat on the increase in the number of microorganisms that cause spoilage of products and being criteria for meat hygiene and biological safety. As a result of the study, there were no detecting bacteria of the genus *Salmonella* in a sample of 25g of chilled, defrosted, and re-defrosted turkey meat. Also, there was no detecting growth of *Listeria monocytogenes* and bacteria of the *Escherichia coli* group in a sample of 1g of the product. On the other hand, significant changes were in the dynamics of microbial contamination in terms of the number of mesophilic aerobic and facultative anaerobic microorganisms. Thus, in cooled samples, the total microbial contamination was 3.2×102±0.2 colonies of forming units per 1g of product. In samples of defrosted turkey meat, this indicator increased 1.8 times and amounted to 5.6×102±0.4. However, the obtained value did not exceed the maximum permissible, regulated in the normative and technical documentation. Samples of re-defrosted turkey meat in terms of quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms corresponded to 4.7×103±0.2, which is 14.5 times higher than in chilled meat samples and 8.4 times higher than in defrosted meat. In addition, the established value of the indicator of microbial contamination in re-defrosting meat exceeded the maximum permissible value established by regulatory enactments. Thus, there is a direct effect of repeated freezing of raw meat on its good quality and safety. Therefore, the solution to such a practical problem as identifying the thermal state of turkey meat acquires a significant role.

**Keywords:** Contamination, Meat hygiene, Meat spoilage, Microbiological control, Storage.

## INTRODUCTION

The most important strategic task of the state is to ensure the food safety of products on the market. For its high nutritional and biological value, Turkey meat is gaining popularity among consumers, but, at the same time, the role and need to control the bio-safety criteria of the raw materials produced increases (Ismail and Joo, 2017; Lauritsen et al., 2019). The slaughter products received and put into circulation from sick poultry, produced with non-observance of veterinary and sanitary requirements, can threaten human health concerning pathogenic and opportunistic microorganisms (Carrasco et al., 2012; Tsigarida et al., 2019). When controlling the products sold, it is necessary to consider the complexity of methods and veterinary and sanitary requirements, regulated by regulatory documents, to the good quality and safety of food products, its identification characteristics at each stage of circulation (Salinas et al. 2014; Orlova and Drozd, 2020).

Demand increase for this product type leads to production intensification and appearance of an effect on the shelves in a wide variety and quantity (Colmenero, 2000). That does not exclude the occurrence of cases of counterfeit turkey meat products or a decrease in its quality (Galarz et al., 2010). However, turkey meat can easily undergo spoilage during storage during trading because of non-compliance with the conditions of its production, temperature violation, storage humidity conditions, or transportation (Bolder, 2007; Voidarou et al., 2011; Chakchouk-Mtibaa et al., 2017). Under improperly storage conditions, turkey meat develops autolytic processes and these processes lead to structural changes in muscle tissue under intensive development of lactic acid, putrefactive microorganisms, micrococci, yeast, molds, etc (Díaz et al., 2008; Luong et al., 2020). As a result, the meat's biochemical composition changes, and protein breakdown products accumulate (Rouger et al., 2017; Saewan et al., 2021). There are a lot of methods and ways for extending poultry meat shelf life that significantly reduce the microflora growth, thereby ensuring the products bio-safety (Patterson and Gibbs, 1973; Mahmoud et al., 2021). Such methods are high pressure freezing, specialized packaging, modified media, etc (Yuste et al., 2002).

In this connection, veterinarians in large companies representing the state and industrial veterinary service in food markets, processing, and refrigeration enterprises, should pay special attention to quality control of raw meat, including the indicators of good quality and biological safety (Chousalkar et al., 2019; Williams et al., 2020).



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The requirements of regulatory and technical documents established the conditions, storage periods, and quality parameters of turkey meat (Yang et al., 2021).

However, to make a profit, unscrupulous manufacturers can use expired turkey meat to manufacture culinary products intended for direct consumption, which, in turn, can cause poisoning in humans and the development of harmful infections (Carroll and Alvarado, 2008; Zhang et al., 2016).

In cases of non-observance veterinary and sanitary rules for raw meat production and circulation and within the framework of state monitoring, it is required to analyze microbiological safety according to the indicators provided by the Customs Union Technical Regulations 034/2013 "On the safety of meat and meat products" (CUTR 034/2013). These indicators are quantity mesophilic aerobic and facultatively anaerobic microorganisms, bacteria of the *Escherichia coli* group, *Staphylococcus aureus*, *Proteus bacilli*, *Salmonella*, *Listeria monocytogenes* yeast, and mold, sulfite-reducing clostridia (Cai et al., 2019).

According to the regulatory documents (Prylipko, 2019; Moiseeva and Motovilov, 2020), the shelf life of chilled and frozen turkey meat is advisory and set by the manufacturer. Therefore, to prevent the sale of meat in the trading network that does not meet biological safety requirements, it is necessary to assess it in the conditions of actual product circulation.

The study aimed to establish the effect of a single and repeated defrosting of turkey meat on the increase in the number of microorganisms that cause spoilage of products, as well as being criteria for biological safety.

## MATERIALS AND METHODS

Turkey meat samples for experiments were bought in retail stores in St. Petersburg, Russia. The studies established the effect of single and repeated defrosting of turkey meat on the increase in the number of microorganisms that cause spoilage of products and being criteria for biological safety. A total of 128 samples of turkey meat were studied, including thigh = 26 pieces, wing = 31 pieces, drumstick = 18 pieces, breast = 53 pieces (Figure 1).


**Figure 1** - The research objects

For identifying bacteria of the genus *Salmonella*, 25g of ground turkey meat was inoculated on a selective enrichment medium - tetrathionate broth (Müller-Kaufmann) in a ratio of 1:10. Then they were incubated at a temperature of 37±1°C for 24 hours. After that, they were sub-cultured on two differential diagnostic media, on XLD-agar and bismuth-sulfite agar. Then they were incubated into a thermostat (37±1°C). The cultures were counted after 24 hours, and the final one - after 48 hours (ISO 6579-1:2017).

On XLD-agar, bacteria of the genus *Salmonella* formed characteristic colonies with a black center and pink colonies with a dark pink center, or yellow colonies with or without blackening. On bismuth-sulfite *salmonella* agar, black colonies with a metallic sheen were formed. The medium under the colonies was colored, either in the form of greenish colonies with a dark green rim, or colorless colonies without color of the medium.

For identifying Listeria monocytogenes, 25g of ground turkey meat was inoculated into Fraser's broth for selective enrichment in a ratio of 1:9. Then it was incubated at a temperature of 37±1°C for 48 h. After that, they were sub-cultured onto differential diagnostic media: ALOA (Listeria Chromogenic Agar Base Acc. to Ottaviani and Agosti), and listeria nutrient agar, incubated in a thermostat (37±1°C), the inoculations were counted after 24 hours, and the final one - after 48 hours.

On ALOA, listeria grew in blue-green colonies with an opaque rim. On listeria nutrient agar, listeria formed small grayish-yellow colonies with a black halo (GOST 32031-2012).

Determination of bacteria of the *Escherichia coli* group was by sowing a crushed sample of 1g of turkey meat on Kessler's medium. Crops were incubated at 37±1°C for 24 hours. To consider the growth, a change in the color of the medium and the presence of gas bubbles were noted (GOST 31747-2012).

The quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms was determined by inoculation in agar nutrient media. First, a 1g weighed portion of the test meat was minced, and a series of successive dilutions were prepared. For inoculation, dilutions of 1:1000, 1:10000, 1:100000 were taken. The inoculation was done in parallel on two Petri dishes from each dilution. For doing this, 1mL of material, without touching, was pipetted onto the bottom of the dish. Then the material was distributed over the plate with light rotary movements. After this, 15 minutes later, 20 mL of



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defrosted and cooled meat were added peptone agar with a temperature of (45±1)°C. The medium was allowed to solidify and incubated upside down at 30°C for 72 hours. After that, the crops were recorded, and the colonies were counted. The resulting number of colonies was multiplied by the dilution, and then the arithmetic mean was obtained, which was taken as the value of QMAFAnM in CFU/g (GOST 10444.15-94).

The data obtained from observation checklists were analyzed using SPSS version 21.0 and then exported to Microsoft Excel to calculate the various scores. Descriptive analyses used mean, standard deviation, maxima, and minima for each category. Scores were assessed according to food safety. The two-sample t-test was used to compare microbiological safety Indicators and microbial meat contamination data sets. Comparisons were conducted by fixed-effects analysis of variance. Data were first tested using quantile-quantile plots or the Kolmogorov–Smirnov test, and variance equality was checked using the modified Levene test. Next, Non-normally distributed data sets and those with a sample size less than ten were analyzed using the non-parametric Wilcoxon rank-sum test for two category cases, i.e., sex (male or female), food safety training status (trained or untrained), etc., and the Kruskal-Wallis rank-sum test was used when there were more than two categories. Statistically significant differences were based on 95% confidence limits, i.e., $\alpha = 0.05$ or $p < 0.05$.

## RESULTS AND DISCUSSION

When conducting microbiological studies on the biological safety indicators of turkey meat of various parts of chilled, defrosted, and re-defrosted carcasses, bacteria of the genus *Salmonella* were not detected in 25g of products. Also, in all 25g samples, there was no growth of Listeria monocytogenes. In addition, *Escherichia coli* bacteria had not been detected when inoculating in 1g of the product in all studied samples of turkey (Table 1).

Significant changes were in the dynamics of microbial contamination in terms of the number of mesophilic aerobic and facultative anaerobic microorganisms. Thus, in cooled samples, the total microbial contamination was $3.2\times10^2\pm0.2$ colonies of forming units per 1g of product. In samples of defrosted turkey meat, this indicator increased 1.8 times and amounted to $5.6\times10^2\pm0.4$. However, the obtained value did not exceed the maximum permissible, regulated in the normative and technical documentation. Samples of re-defrosted turkey meat in terms of QMAFAnM corresponded to $4.7\times10^3\pm0.2$, which is 14.5 times higher than in chilled meat samples and 8.4 times higher than in defrosted meat. In addition, the established value of the indicator of microbial contamination in re-defrosting meat exceeded the maximum permissible value established by regulatory enactments.

Considering the general microbial contamination of parts of turkey carcasses separately, it should be noted that the maximum value of the microbial number was observed in the wing, the minimum - in the breast (Table 2). The higher microbial contamination of wing meat can be explained by the larger surface of this part of the carcasses relative to the volume of muscle tissue than the breast and other parts of the carcasses, which contributes to the more active growth of microflora.

Thus, in chilled wing meat, the value of QMAFAnM was $3.6\times10^2\pm0.3$, in defrosted meat - $6.3\times10^2\pm0.3$, which is 1.8 times higher than the value of microbial contamination of cooled samples. At the same time, in the repeatedly defrosted turkey wing meat, the microbial number was $5.8\times10^3\pm0.1$, which exceeds this indicator in chilled meat by 16.1 times, in defrosted meat, by 9.2 times. On the other hand, the smallest value of the index of microbial contamination was established in chilled breast meat - $2.5\times10^2\pm0.4$, which significantly increased in defrosted samples by 1.9 times and amounted to $4.9\times10^2\pm0.2$, in re-defrosted samples - in 14.8 times relative to chilled meat and 7.6 times relative to defrosted meat and amounted to $3.7\times10^3\pm0.2$ ($p<0.05$).

**Table 1** – Turkey meat microbiological safety Indicators

| Indicator | Chilled Turkey Meat | Defrosting turkey meat | Re-defrosted turkey meat |
|---|---|---|---|
| *Salmonella* bacteria | Not detected in 25g | Not detected in 25g | Not detected in 25g |
| *Listeria monocytogenes* | Not detected in 25g | Not detected in 25g | Not detected in 25g |
| *Escherichia coli* bacteria | Not detected in 1g | Not detected in 1g | Not detected in 1g |
| QMAFAnM, CFU/g (average value) | $3.2\times10^2\pm0.2$ | $5.6\times10^2\pm0.4$ | $4.7\times10^3\pm0.2$ |

M±m, n=128; p<0,05

**Table 2** - Turkey meat microbial contamination (QMAFAnM), CFU/g

| Part of the carcass | Chilled Turkey Meat | Defrosting turkey meat | Re-defrosted turkey meat |
|---|---|---|---|
| Thigh[1] | $3.4\times10^2\pm0.3$ | $5.8\times10^2\pm0.4$ | $3.8\times10^3\pm0.3$ |
| Drumstick[2] | $3.3\times10^2\pm0.2$ | $5.4\times10^2\pm0.1$ | $5.5\times10^3\pm0.4$ |
| Breast[3] | $2.5\times10^2\pm0.4$ | $4.9\times10^2\pm0.2$ | $3.7\times10^3\pm0.2$ |
| Wing[4] | $3.6\times10^2\pm0.3$ | $6.3\times10^2\pm0.3$ | $5.8\times10^3\pm0.1$ |

M±m, $n^1=26$, $n^2=18$, $n^3=53$, $n^4=31$; p<0,05



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As a result of studying the microbial contamination of chilled, defrosted, and re-defrosted turkey meat, it was found that single freezing and thawing of meat affects the microbial contamination of products. Still, the obtained values do not exceed the maximum permissible values and consistent with the works of Doulgeraki et al. (2012) and Yu et al. (2021), which describe the positive effect of freezing on extending the shelf life of poultry meat by reducing the activity of microorganisms. However, repeated low-temperature processing of turkey meat and its longer storage contribute to a sharp increase in microbial contamination, including in violation of the requirements for the indicator of the number of mesophilic aerobic and facultative anaerobic microorganisms, which is consistent with the works of Saewan et al. (2021) and Mahmoud et al. (2021). It should be noted that single and double defrosting of turkey meat does not lead to the growth of pathogenic microorganisms that constitute the criteria for biological safety - bacteria of the genus *Salmonella*, *L. monocytogenes*, and bacteria of the *Escherichia coli* group, which is also reflected in the works of Yu et al. (2021) and Mahmoud et al. (2021).

According to Gram, the microbiological study results were also confirmed by microscopy of smears-prints prepared from meat thickness and stained. Looking at least 25 fields of view in smears-prints made from chilled and defrosted meat samples, microorganisms were not detected, or single cocci and/or sticks were recorded, there were no signs of muscle tissue decay. However, in smears-prints from samples of repeatedly defrosted meat in the microscope's field of view, 22±2 microbial cells and traces of muscle tissue decay in the form of destructively altered muscle fibers were established.

The presence of an increased number of microorganisms detected in smear-prints from re-defrosted meat, as well as the excess in the corresponding samples of the permissible value of QMAFanM, is explained by high proportion of destroyed muscle fibers, disruption of the integrity of the sarcolemma, and going beyond the muscle cells of the sarcoplasm, which together contributes to the development of microflora (Figure 2). The structure of the muscle tissue of defrosted meat is moderately disturbed, single ruptures of muscle fibers are observed, which does not lead to the intensive development of microorganisms (Figure 3). While in chilled meat, while maintaining the integrity of muscle fibers and the absence of destructive changes (Figure 4), the index of microbial contamination meets safety requirements.

**Figure 2 -** Micro-picture of the native preparation of repeatedly defrosted turkey meat, magnification 10×4

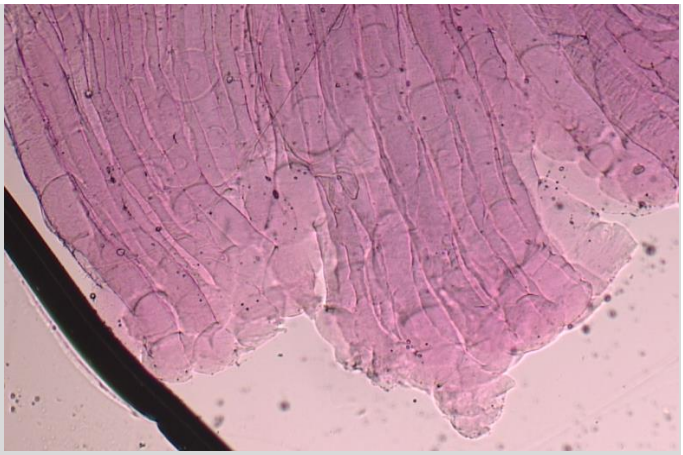

**Figure 3 -** Micro picture of a native preparation of defrosted turkey meat, magnification 10×4

**Figure 4 -** Micro-picture of a native preparation of chilled turkey meat, magnification 10×4



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## CONCLUSION

From the foregoing, it follows that there is a direct effect of repeated freezing of raw meat on its good quality and safety. Therefore, the solution to such a practical problem as identifying the thermal state of turkey meat acquires a significant role. Carrying out microbiological studies, the significant dynamics of the total microbial contamination by mesophilic aerobic and facultative anaerobic microorganisms should be noted. With repeated freezing and defrosting of meat, there was a sharp increase in microbial cells compared to the original chilled material by 14.5 times. This value exceeds the permissible value established in the regulatory and technical documents by 4.7 times. This phenomenon is provoked by a significant breakdown of muscle tissue elements, thereby creating a more favorable environment for the development of microorganisms and, as a result, the appearance of initial signs of meat spoilage in violation of safety requirements.

## DECLARATIONS

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**Authors' contribution**
Orlova D, conceived of the presented idea. Kalyuzhnaya T, developed the theory and performed the computations. Stekolnikov A, verified the analytical methods. Kuznetsov Y, encouraged Drozd A, to investigate and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

**Conflict of Interests**
The authors have not declared any conflict of interests.

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# THE EFFECT OF THE PANDEMIC ON THE CONSUMPTION OF ANIMAL PRODUCTS: THE CASE OF KAFKAS UNIVERSITY OF TURKEY

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RESEARCH ARTICLE

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**ABSTRACT**: The COVID-19 pandemic has brought about some changes in consumption preferences and nutritional habits as well as lifestyles of individuals, such as education, working system, and social relationships, especially due to having to stay at home. In this study, the change in animal food consumption dispositions of students who had to stay at home during the pandemic and received online education was examined compared to the pre-pandemic period. In the study, since face-to-face teaching-learning activities were suspended, an online questionnaire was used to collect the study data from a total of 380 students registered at Kafkas University. The average monthly income of the participants and the share they allocated to the total food and animal products expenditures in the budget were determined as 539.64±21.00$, 132.73±5.08$, and 62.18±2.67$, respectively. Although the annual chicken meat consumption amount did not change according to the income levels of the students, it was determined that the consumption of animal products, such as beef, mutton-lamb, milk-yogurt, cheese, and eggs increased as the income level increased. During the pandemic period, the food consumption of 55.3% of the participants and the animal products consumption of 35.8% increased. On the other hand, it was determined that the consumption of food and animal products increased as the income level increased during the pandemic compared to the pre-pandemic period. During the pandemic period, it was determined that red meat and salami-sausages consumption of 31.8% of the students decreased by 37.90% and 42.15%, respectively, but that the chicken meat consumption of 31.3% of the students and milk and dairy products consumption of 37.9% increased by 31.02% and 39.17%, respectively. As a result, it was determined that there were significant changes in the nutritional habits of the students during the COVID-19 pandemic, and it was determined that there were significant increases in the consumption of animal products other than red meat. The primary reason why red meat consumption did not increase is thought to be due to the high price of the product compared to consumers' income levels.

**Keywords:** Animal Products, Consumption, COVID-19, Meat, Pandemic.

## INTRODUCTION

The pandemic, which was caused by the coronavirus and called the COVID-19 pandemic, was first seen in December 2019. The World Health Organization (WHO) declared COVID-19 as a high-risk global pandemic across the world on March 12, 2020 (Andrews et al., 2020). The first case in Turkey was reported on March 11, 2020, and measures were taken across the country gradually due to the increase in the number of cases. The most important measure taken was lockdowns. In this context, a partial and/or full closure was put into effect across the country as of April 29, 2020 (TR Ministry of Health, 2020).

The COVID-19 pandemic has brought about several changes in consumption preferences and nutritional habits as well as lifestyles of individuals, such as education, working system, and social relationships, especially due to having to stay at home (Dilber and Dilber, 2020; Ersoy and Yardimci, 2020; Taskin et al., 2020). The concept of healthy eating has come to the fore in society to protect against COVID-19 and improve the immune system (Andrews et al., 2020; Saul, 2020; Zhang and Liu, 2020). A healthy diet can be achieved by an adequate and balanced intake of nutrients that meet the energy needs of the body. When nutrients are not taken adequately or taken more or less than necessary, growth and development will stop and the health of the person will gradually deteriorate (Dilber and Dilber, 2020). Nutrition does not only relate to health, but it is also a strategic issue. As a matter of fact, a country needs physically and mentally strong, healthy, and talented individuals to reach the desired social and economic level of civilization, which is closely related to balanced and healthy nutrition (Akin et al., 2019; Dilber and Dilber, 2020; Saul, 2020; Demir Ayvazoglu and Aydin, 2021). In this context, university students are at a critical age period, when their future eating habits are settled. In the study, the change in animal food consumption dispositions of students who had to stay at home during the pandemic and received online education was examined compared to the pre-pandemic period. The study was conducted to evaluate the nutritional habits of individuals in society during the pandemic period through the students of Kafkas University.



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## MATERIALS AND METHODS

### Materials

A total of 20.850 students were registered at Kafkas University in Kars, Turkey as of January 2021. It was not possible to reach the entire population; therefore, we decided to take a sample. The sample size was determined as at least 378 individuals based on a 5% margin of error and 95% confidence interval. Since face-to-face education was suspended due to the COVID-19 pandemic, the study data were collected by using an online questionnaire form created on docs.google.com. The questionnaire was applied to a total of 400 students between February 7 and March 31, 2021, considering that there may be reasons limiting the study, such as missing data. Eventually, 380 questionnaires were included in the study due to missing data on some questionnaires.

### Methods

Research methods consist of research design, data access, data analysis, and evaluation.

### Data analysis

The analysis of the study data was conducted on the SPSS software package (Version: 23.0; IBM, USA). Descriptive statistics of variables, such as demographic information and nutritional habits of the research group, were performed and presented in tables. While doing the analysis and interpretation of the data, the Chi-square test was used to analyze categorical data, and the One Way ANOVA test (one-way analysis of variance) was employed to determine the relationships between monthly household income and animal products expenditures and consumption amounts. Tukey test was used to determine the differences between the groups, and $P<0.05$ was taken as the level of significance.

## RESULTS AND DISCUSSION

Consumption of animal products is one of the basic needs for a balanced and healthy diet and is indispensable for the development of society and economy and the sustainability of development. In this respect, easy access to animal products at affordable prices and quality is the most important goal of developed and developing countries, but access to such healthy foods by all segments of society cannot be provided at an affordable price and in adequate quantity (Akin et al., 2020). Some demographic data of the students participating in the study are given in Table 1.

As seen in Table 1, 46.6% of the participants were male, 53.4% were female, 52.9% came from the provinces in the Eastern and Southeastern Anatolia Regions, the fathers of the 58.9% were illiterate or primary/secondary school graduates. The data about the monthly household income level of the students and their monthly animal product expenditures are given in Table 2.

As seen in Table 2, the expenditures of the participants on milk and dairy products did not change significantly according to their income level, whereas the share allocated to animal products, namely red meat, chicken meat, and fish, especially by those with a monthly income level above $900, increased, which was statistically significant ($P<0.001$). This situation can be explained by “Engel's Law”. As a matter of fact, red meat is characterized by high production costs and high output prices compared to other food products and can be considered a luxury food item (Charlebois et al., 2016; Aktas, 2020). In this context, as the income level increases, the demand for red meat increases, as well. Parallel to the results of the current study, many studies have reported that there is a significant relationship between the average monthly household income groups and the quantity of animal products purchased (Celik and Sengul, 2001; Sengul, 2002; Seker et al., 2011).

It was determined that the average monthly income level of the participants was 539.64±21.00$ (X±SEM), they spent 25% (132.73±5.08$) of their budget on food, and that 46.85% (62.18±2.67$) of their food expenditure was allocated to animal products. According to the data obtained from the survey, of the monthly animal product expenditures, 19.99% was allocated to red meat, 13.30% to chicken meat, 6.76% to fish, and 6.79% to milk and dairy products.

The household income levels of the students and their annual consumption of animal products are given in Table 3. As seen in Table , the students participating in the study consumed an average of 9.31 kg of beef, 5.89 kg of mutton-lamb, 16.94 kg of chicken meat, 37.38 kg of milk-yogurt, 20.84 kg of cheese, and 168.27 eggs per capita per year. In the study, it was determined that 75% of the students did not consume milk regularly, so the quantity of milk and yogurt consumption was evaluated together. In addition, the students’ annual consumption of chicken meat did not change significantly according to their income levels; however, the annual consumption amount of animal products, namely, beef, mutton ($P<0.05$), milk-yogurt and eggs, was lower in those with a monthly income of ≤300$ than the average and higher in those with a monthly income of ≥900$ than the average, and this difference was statistically significant ($P<0.001$). This situation can be explained by the high "Income Elasticity of Demand" in animal products, especially red meat, in all economic strata in Turkey. As a matter of fact, it can be said that the consumption shows a tendency towards red meat with the increase in income. In parallel with the results of the study, some studies have shown that there is a positive relationship between income level and red meat consumption (Aktas and Hatirli, 2010; Uzunoz and Karakas, 2014; Aktas, 2020).



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**Table 1** - Some demographic data of the participants in present study.

| Parameter | | Frequency | Percent |
|---|---|---|---|
| Gender | Male | 177 | 46.6 |
| | Female | 203 | 53.4 |
| | Total | 380 | 100.0 |
| Geographical region where the student lives | Mediterranean | 32 | 8.4 |
| | Eastern Anatolia | 138 | 36.3 |
| | Aegean | 33 | 8.7 |
| | Southeast | 63 | 16.6 |
| | Central Anatolia | 41 | 10.8 |
| | Black Sea | 26 | 6.8 |
| | Marmara | 34 | 8.9 |
| | Abroad | 13 | 3.4 |
| | Total | 380 | 100.0 |
| Education level of the father | Primary school | 144 | 37.9 |
| | Middle school | 72 | 18.9 |
| | High school | 88 | 23.2 |
| | University | 68 | 17.9 |
| | Not literate | 8 | 2.1 |
| | Total | 380 | 100.0 |

**Table 2** - Students' monthly household income levels and their monthly food and animal product expenditures ($)

| Product expenditures | Income level* | N | Mean | Std. Error | Minimum | Maximum | F/P value |
|---|---|---|---|---|---|---|---|
| Total food expenditures | ≤300 $ | 112 | 81.44[a] | 3.73 | 12.03 | 240.67 | F=99.403 P=0.000 P<0.001 |
| | 301-600 $ | 136 | 109.87[b] | 4.71 | 12.03 | 240.67 | |
| | 601-900 $ | 80 | 141.65[c] | 9.38 | 24.07 | 421.18 | |
| | ≥901 $ | 52 | 289.25[d] | 18.79 | 18.05 | 722.02 | |
| | Total | 380 | 132.73 | 5.08 | 12.03 | 722.02 | |
| Red meat expenditures | ≤300 $ | 112 | 16.42[a] | 1.45 | .00 | 72.20 | F=21.614 P=0.000 P<0.001 |
| | 301-600 $ | 136 | 22.75[a] | 2.317 | .00 | 180.51 | |
| | 601-900 $ | 80 | 23.92[a] | 1.764 | .00 | 60.17 | |
| | ≥901 $ | 52 | 63.72[b] | 11.59 | 6.02 | 601.68 | |
| | Total | 380 | 26.53 | 2.01 | .00 | 601.68 | |
| Chicken meat expenditures | ≤300 $ | 112 | 14.74[a] | 1.28 | .00 | 60.17 | F=8.390 P=0.000 P<0.001 |
| | 301-600 $ | 136 | 16.94[a] | 1.26 | .00 | 60.17 | |
| | 601-900 $ | 80 | 17.40[a] | 1.30 | .00 | 84.24 | |
| | ≥901 $ | 52 | 26.19[b] | 2.07 | 4.81 | 90.25 | |
| | Total | 380 | 17.66 | 0.73 | .00 | 90.25 | |
| Fish expenditures | ≤300 $ | 112 | 5.94[a] | 0.57 | .00 | 36.10 | F=22.158 P=0.000 P<0.001 |
| | 301-600 $ | 136 | 7.44[a] | 0.75 | .00 | 60.17 | |
| | 601-900 $ | 80 | 9.92[b] | 0.88 | .00 | 36.10 | |
| | ≥901 $ | 52 | 18.03[c] | 2.16 | .00 | 60.17 | |
| | Total | 380 | 8.97 | 0.51 | .00 | 60.17 | |
| Milk and dairy products expenditures | ≤300 $ | 112 | 8.97[a] | 0.56 | .00 | 24.07 | F=0.33 P=0.804 P>0.05 |
| | 301-600 $ | 136 | 9.08[a] | 0.44 | 1.20 | 30.08 | |
| | 601-900 $ | 80 | 8.61[a] | 0.50 | 2.41 | 18.05 | |
| | ≥901 $ | 52 | 9.51[a] | 0.64 | 3.01 | 24.07 | |
| | Total | 380 | 9.01 | 0.26 | .00 | 30.08 | |

*1 $= 8.31 TL (Date: September 07, 2021/TCMB [The Central Bank of the Republic of Turkey], 2021), [a,b,c,d]: The difference between the mean values shown with different letters according to income groups for the same parameter is statistically significant.



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**Table 3 -** Monthly household income level of the students and the annual consumption of animal products per capita (kg/piece)

| The animal products consumed* | Income level | Mean | Std. Error | F/P value |
|---|---|---|---|---|
| **Beef** | ≤300 $ | 6.03[a] | 0.89 | F=16.740 P=0.000 P<0.001 |
| | 301-600 $ | 9.35[a] | 1.08 | |
| | 601-900 $ | 11.20[b] | 1.40 | |
| | ≥901 $ | 15.59[c] | 2.29 | |
| | Total | 9.31 | 0.67 | |
| **Mutton-lamb** | ≤300 $ | 4.25[a] | 0.66 | F=2.809 P=0.049 P<0.05 |
| | 301-600 $ | 5.88[a] | 0.88 | |
| | 601-900 $ | 4.78[a] | 0.86 | |
| | ≥901 $ | 9.00[b] | 1.57 | |
| | Total | 5.89 | 0.46 | |
| **Chicken meat** | ≤300 $ | 15.59[a] | 1.60 | F=0.114 P=0.952 P>0.05 |
| | 301-600 $ | 16.37[a] | 1.45 | |
| | 601-900 $ | 16.27[a] | 1.72 | |
| | ≥901 $ | 19.57[a] | 2.43 | |
| | Total | 16.94 | 0.85 | |
| **Milk-yogurt** | ≤300 $ | 29.55[a] | 2.01 | F=12.320 P=0.000 P<0.001 |
| | 301-600 $ | 38.82[b] | 1.72 | |
| | 601-900 $ | 38.27[b] | 2.00 | |
| | ≥901 $ | 48.94[c] | 2.19 | |
| | Total | 37.38 | 1.04 | |
| **Cheese** | ≤300 $ | 15.34[a] | 1.19 | F=12.493 P=0.000 P<0.001 |
| | 301-600 $ | 22.27[b] | 1.07 | |
| | 601-900 $ | 22.49[b] | 1.21 | |
| | ≥901 $ | 26.23[b] | 1.50 | |
| | Total | 20.84 | 0.64 | |
| **Eggs (unit)** | ≤300 $ | 82.15[a] | 18.36 | F=7.949 P=0.000 P<0.001 |
| | 301-600 $ | 180.52[b] | 19.25 | |
| | 601-900 $ | 188.12[b] | 19.93 | |
| | ≥901 $ | 217.32[b] | 25.20 | |
| | Total | 168.27 | 10.60 | |

*: The data of the participants who stated that they never consumed the product were not included in the calculation of the average value. [a,b,c]: The difference between the mean values shown with different letters according to income groups for the same parameter is statistically significant.

According to the Agricultural Products Market Report, 13.3 kg of beef is consumed per capita in Turkey (TEPGE, 2021). Although Turkey is above the world average (6.4 kg) in beef consumption, it falls behind many countries, such as Argentina, Australia, the US, European Union, Russia, Israel, in total red meat consumption due to the high consumption of pork in many developed and developing countries (OECD, 2021). One of the main reasons for the low consumption of red meat in Turkey is the high production costs and price compared to other foods (Taskin et al., 2020).

It was determined that the students in the study consumed an average of 15.20 kg of red meat (9.31 kg of beef and 5.89 kg of mutton-lamb) annually. It is thought that one of the most important reasons why this finding was lower than beef consumption (13.3 kg) and higher than mutton-lamb consumption (1.5 kg) per capita in Turkey is that 52.9% of the students in the study lived in provinces in the Eastern and Southeastern Anatolia Regions (TEPGE, 2021). As a matter of fact, considering the geographical location of the provinces, some studies have indicated that people in the provinces of the Eastern and Southeastern Anatolia Regions often consume red meat and that they mostly prefer mutton (Karakus et al., 2008; Tosun and Hatirli, 2009; Seker et al., 2011; Kara et al., 2020).

In the study, it was found that the students' annual consumption of chicken meat and mutton did not change significantly, except for those with a monthly income of ≥$900. Similarly, a study conducted in Gaziantep showed that regardless of the difference between income levels, income subgroups also consumed mutton at rates close to each other (Karakus et al., 2008).



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Chicken meat is widely consumed in Turkey due to its cooking time, ease of cooking, and more affordable price than red meat (BESD-BIR, 2021). According to the 2020 data of BESD-BIR (2021) compared to the total world poultry meat production of 132 million tons, Turkey ranks in the top 10 countries in the world with a production of 2 million 194 thousand tons, and the annual per capita consumption of poultry meat is 21.10 kg. In the study, it was evaluated that the students consumed chicken meat below the Turkey average with an annual average consumption of 16.94 kg of chicken meat. The amount of chicken meat consumption in the study was found to be higher than the findings of the studies conducted in Kırşehir, Amasya, and Sivas provinces (Kizilaslan and Nalinci, 2013; Karadavut and Taskin, 2014; Bircan et al., 2017) and lower than the findings of the studies conducted in Kahramanmaraş, Bingöl, and Yozgat provinces (Karakaya and Inci 2014; Tumer et al., 2016; Eleroglu et al., 2018). This can be explained by the fact that the students consumed relatively more mutton than the Turkey average (TEPGE, 2021).

In the study, it was calculated that the students consumed an average of 168 eggs per year, and this value remained at a lower level than the overall average of Turkey, which was reported as 214 eggs per year (YUM-BIR, 2018). The difference between the average annual egg consumption per capita in Turkey and the research data is thought to be due to the inclusion of indirect consumption (cake, biscuit, etc.) in the average annual consumption value in Turkey. On the other hand, the average annual egg consumption per capita reported in the studies conducted in Yozgat (Eleroglu et al., 2018), Bingöl (Inci et al., 2014), Sivas (Bircan et al., 2017), and Uşak (Parlakay et al., 2017) provinces as 130.49, 146.3, 123.4, and 102, respectively, was lower than the average annual egg consumption per capita calculated in the present study. In line with these findings, it can be said that the egg, which is known as the cheapest protein source, is widely consumed among students.

Today, there is an increase in the demand for milk and dairy products due to the increase in awareness and income level. However, in the study, it was determined that the majority of the students (about 75%) did not consume milk regularly and preferred yogurt and cheese more. Similarly, some studies in the literature have reported that students do not have regular milk consumption habits (Engindeniz et al., 2021).

In the study, it was determined that the students consumed an average of 37.38 kg of milk and yogurt per capita per year. The annual per capita consumption of yogurt was found as 32.84 kg by Engindeniz et al. (2021), 31.96 kg by Karakaya and Akbay (2013), and 27.66 kg by Andic et al. (2002). It is thought that the finding of the present study was high due to the inclusion of milk consumption in the average annual consumption per capita in the study. On the other hand, Savran et al. (2011) reported average milk consumption as 64 l/year and yogurt consumption as 55 kg/year.

In the study, the average annual cheese consumption of the participants was determined as 20.84 kg. This value was above the average annual cheese consumption amount (17.5 kg) per capita in Turkey in 2019 (USK, 2020). Although this difference is thought to be regional, it can be said that cheese and yogurt consumption is common in the provinces in the Eastern and Southeastern Anatolia Regions. On the other hand, the average annual cheese consumption per capita was reported as 18.48 kg by Engindeniz et al. (2021), 23 kg by Savran et al. (2011), 14.65 kg by Karakaya and Akbay (2013), and 17.63 kg by Andic et al. (2002).

The reasons for consuming red meat and chicken meat by the students are given in Table 4. As seen in Table 4, being healthy-nutritious ranks first among the reasons why the participants consumed red meat. This is followed by being tasty, habit, and availability, respectively. Among the reasons for consumption of chicken meat, being healthy and tasty rank first and second place, respectively, and low price ranks the third place. Similar to the results of this study, Karakus et al. (2008) found being nutritious as the first ranking characteristic, and Seker et al. (2011) determined being tasty as the most prominent parameter. In line with these data, it can be said that the participants had an awareness of consuming animal products in that they found them both healthy and tasty.

The ranking of the participants' preference for some animal products is given in Table 5.

As seen in Table 5, the red meat products that students preferred most were minced, flaked, and bony and the most preferred three chicken products were whole chicken, breast meat, and chicken legs, respectively. In addition, the most preferred milk types were organic milk, pasteurized, and long-life milk, and the most preferred egg types or common reasons for buying eggs included affordable price, organic eggs, and cage-free chicken eggs, respectively.

According to the findings, the primary red meat products that students preferred were minced meat, flaked meat, and meat with bones. In parallel with this research finding, Taskin et al. (2020) reported similar product rankings. In the study, it can be said that the consumption of minced meat as a red meat product more than other red meat preparations was because minced meat was used in various types of food and its price was affordable. In the study, it was found that the chicken products that students preferred most were whole chicken and breast meat and that the findings were similar to those of other studies (Iskender et al., 2015; Kara et al., 2020). It is thought that the tendency of the participants towards cheap products in chicken meat was related to their income level.

In the study, it was determined that 7.10% of the participants did not consume red meat, 2.37% did not consume chicken meat, 1.58% did not consume milk, and that 2.37% did not consume eggs. In parallel with the results of the study, Sancak and Basat Dereli (2019) determined that 7.6% of their participants did not consume meat, and this rate was found as 6% by Kusat and Sahan (2021). On the other hand, according to a report of the Ministry of Health of the Republic of Turkey and Hacettepe University (2014), 20.2% of the population does not consume red meat. Differences in red meat consumption can be explained by the change of consumption habits according to regions and urban-rural settlements. The rate of those who do not consume other animal products is between 1-3%, which is considered as a positive development in terms of health.



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**Table 4 -** Reasons for consuming red meat and chicken meat

| Parameter | Reasons for consuming red meat | | Reasons for consuming chicken meat | |
|---|---|---|---|---|
| | Frequency | Ranking | Frequency | Ranking |
| Healthy- nutritious | 276 | 1 | 194 | 1 |
| Tasty | 170 | 2 | 140 | 2 |
| Habit | 75 | 3 | 76 | 5 |
| Availability | 34 | 4 | 109 | 4 |
| Low price | 0.0 | - | 136 | 3 |
| Easy to cook | 0.0 | - | 58 | 6 |

**Table 5 -** The ranking of the participants' preference for some animal products

| The most preferred products when buying red meat | | | The most preferred products when buying chicken meat | | |
|---|---|---|---|---|---|
| **Product** | **Frequency** | **Ranking** | **Product** | **Frequency** | **Ranking** |
| Minced | 225 | 1 | Whole chicken | 158 | 1 |
| Flaked | 154 | 2 | Breast meat | 144 | 2 |
| Bony | 94 | 3 | Chicken legs | 124 | 3 |
| Sausages-salami | 76 | 4 | Wings | 111 | 4 |
| Tenderloin, chops | 72 | 5 | Drumsticks | 52 | 5 |
| Lamb neck | 24 | 6 | Chops | 42 | 6 |
| No consumption | 27 | 7.10% | No consumption | 9 | 2.37% |
| **The most preferred milk type or common reasons for buying milk** | | | **The most common reasons for buying eggs** | | |
| Product | **Frequency** | **Ranking** | **Product** | **Frequency** | **Ranking** |
| Organic milk | 221 | 1 | Affordable | 122 | 1 |
| Pasteurized | 129 | 2 | Organic | 114 | 2 |
| Long life (UHT) | 113 | 3 | Cage-free chicken eggs | 66 | 3 |
| Milkman delivery | 79 | 4 | Size | 20 | 4 |
| Affordable | 64 | 5 | Color | 10 | 5 |
| Any type | 12 | 6 | Any type | 81 | 6 |
| No consumption | 6 | 1.58% | No consumption | 9 | 2.37% |

The rate of change in food and animal product consumption during the pandemic compared to the pre-pandemic period is given in Table 6.

As seen in Table 6, the total food consumption of 55.3% of the participants and the animal products consumption of 35.8% of them increased during the pandemic. In the study, it was observed that the consumption of food and animal products increased as the income level of the participants increased during the pandemic compared to the pre-pandemic period, and the difference between the terms was statistically significant ($P<0.001$). In line with the research findings, Naja and Hamadeh (2020) stated that a protein-rich diet that is also rich in fresh fruits and vegetables was necessary to keep the infection away and support the immune system.

In the study, those who stated that their consumption of animal products increased attributed this increase to regular and balanced nutrition at home (47.06%), additional eating to increase body resistance (41.18%), and restaurants that were closed during lockdowns (11.76%). Those who stated that their consumption of animal products decreased attributed this decrease to the decrease in household income level (64.71%) and other reasons (35.29%).

The changes and rates of consumption of some animal products during the pandemic are given in Table 7.

As can be seen in Table 7, there was no change in the amount of animal products consumption between 42.1% and 49.2%, although it varied according to the participants' parameters. In the study, it was determined that the red meat and salami-sausages consumption of 31.8% of the students decreased by 37.90% and 42.15%, respectively. Similarly, Taskin et al. (2020) reported that students' red meat consumption decreased by 56.4% compared to the previous year. Ersoy and Yardimci (2020) reported that the COVID-19 pandemic negatively affected nutrition, especially in low- and middle-income groups.

It was determined in the current study that the chicken meat consumption of 31.3% of the participants and milk and dairy products consumption of 37.9% increased by 31.02% and 39.17%, respectively, during the pandemic. This result was close to the results of Unal et al. (2020) who reported that more food was cooked during quarantine times and that the time allocated for cooking and the types of food that were cooked increased. Similarly, Dilber and Dilber (2020) found that the number of snacks and main meals that individuals consumed increased and that the most consumed foods were pastries and meat and meat products. On the other hand, in his study in which students' eating habits during the pandemic were evaluated, Erdoğan (2021) reported that 45.5% of the students had changes in their eating habits, 50.6% consumed two main meals a day, and 63.6% had breakfast regularly. Kriaucioniene et al. (2020) found that during the



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COVID-19 quarantine period in Lithuania, participants ate more food than usual at home, snacked more, and cooked at home more often.

It can be said that the new normal lifestyle and rules have emerged in Turkey during the coronavirus pandemic, along with the structural and social changes that include education, working life, social relationships, and consumption preferences (Unal et al., 2020). During the lockdowns in the pandemic, authorities in the broadcasts and television programs recommended paying attention to adequate and balanced nutrition, consuming animal proteins such as meat and fish at least twice a week, and preferring healthy foods to reduce the sensitivity and long-term complications caused by the coronavirus (Butler and Barrientos, 2020; Unal et al., 2020).

**Table 6 -** Change in food and animal product consumption during the pandemic compared to the pre-pandemic period

| Income groups | Food consumption in the pandemic | | | Animal product consumption in the pandemic | | | Total |
|---|---|---|---|---|---|---|---|
| | Increased | Decreased | No change | Increased | Decreased | No change | |
| ≤300 $ | 55 | 35 | 22 | 24 | 50 | 38 | 112 |
| | 49.1% | 31.2% | 19.6% | 21.4% | 44.6% | 33.9% | 100.0% |
| 301-600 $ | 71 | 36 | 29 | 52 | 34 | 50 | 136 |
| | 52.2% | 26.5% | 21.3% | 38.2% | 25.0% | 36.8% | 100.0% |
| 601-900 $ | 47 | 7 | 26 | 38 | 14 | 28 | 80 |
| | 58.8% | 8.8% | 32.5% | 47.5% | 17.5% | 35.0% | 100.0% |
| ≥901 $ | 37 | 3 | 12 | 22 | 4 | 26 | 52 |
| | 71.2% | 5.8% | 23.1% | 42.3% | 7.7% | 50.0% | 100.0% |
| Total | 210 | 81 | 89 | 136 | 102 | 142 | 380 |
| | 55.3% | 21.3% | 23.4% | 35.8% | 26.8% | 37.4% | 100.0% |
| $X^2$/P value | $X^2$= 26.009 P=0.000 P<0.001 | | | $X^2$= 36.171 P=0.000 P<0.001 | | | |

**Table 7 -** The effect of the pandemic on the consumption level of some animal products

| Status of change | Red Meat Consumption during the Pandemic | | | Chicken Meat Consumption during the Pandemic | | |
|---|---|---|---|---|---|---|
| | Frequency | Percentage | % Variance | Frequency | Percent | % Variance |
| Increased | 99 | 26.1 | 30.55 | 119 | 31.3 | 31.02 |
| Decreased | 121 | 31.8 | 37.90 | 98 | 25.8 | 29.05 |
| No change | 160 | 42.1 | - | 163 | 42.9 | - |
| **Status of change** | **Consumption of Milk and Dairy Products during the Pandemic** | | | **Consumption of Sausages and Salami during the Pandemic** | | |
| | Frequency | Percentage | % Variance | Frequency | Percentage | % Variance |
| Increased | 144 | 37.9 | 39.17 | 72 | 18.9 | 28.62 |
| Decreased | 64 | 16.8 | 21.94 | 121 | 31.8 | 42.15 |
| No change | 172 | 45.3 | - | 187 | 49.2 | - |

## CONCLUSION

In conclusion, it was determined that there were significant changes in the nutritional habits of the students during the COVID-19 pandemic and that there were significant increases in the consumption of animal products other than red meat. The main reason why red meat consumption did not increase is thought to be due to the high price of the product compared to the income of the consumers. In this context, consumption of red meat, not as a luxury product but as a basic need, in every part of society seems possible only if the prices are brought to an affordable level. It is necessary to reduce producer input costs and the number of dealers in the marketing chain so that prices can go down and consumers can buy red meat as much as they want.



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## DECLARATIONS

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E. Aydin and P. Ayvazoglu Demir planned and designed the research and contributed to the collection of online data. P. Ayvazoglu Demir analyzed the data. E. Aydin wrote the manuscript. All authors discussed the results and contributed to the final manuscript.

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# THE POTENTIAL USES OF SILYMARIN, A MILK THISTLE (*Silybum Marianum*) DERIVATIVE, IN POULTRY PRODUCTION SYSTEM

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*Supporting Information*

REVIEW

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**ABSTRACT**: Due to recent intensive poultry production, there is a great demand to use natural alternative feed additives. One of these alternatives is phytobiotics. Milk thistle (*Silybum marianum*) is a plant that has been used for many years as a natural remedy for the liver diseases. Silymarin is the major dried extract of milk thistle. Silymarin has many flavonolignans that showed antioxidant, anti-inflammatory, anti-fibrotic, anti-lipid peroxidative, immune stimulant, and hepatic cells stabilizing effects. In poultry production system, silymarin has been used in broilers as a growth promotor and in layers to improve the egg quantity and quality. It has been also used as a hepatotonic substance as a result of a potent antioxidant activity. The carcass trait showed improvement after treatment of broilers with silymarin. In addition, enhancement of the immune system and the intestinal health has been detected after application of silymarin in poultry diets. Accordingly, this review article aims to show the different potential uses of silymarin in poultry production system regarding its effect on production performance, antioxidant status, carcass traits, immune response, and intestinal health.

**Keywords:** Antioxidant, Carcass trait, Immunity, Intestinal health, *S. marianum*

## INTRODUCTION

As a result of COVID-19 crisis and drastic decrease in the feed supply, search for alternative feed supplies becomes very urgent (Hafez and Attia, 2020). Moreover, it is important to decrease the need to include antibiotics in the nutrition of poultry to avoid the adverse effects of resistance and the harmful residues in poultry products (Castillo-Lopez et al., 2017). Addition of phytobiotics to poultry feed is regarded as an effective alternative approach. Phytobiotics can improve the nutrient digestibility and the function of birds via increasing the secretion of digestive enzymes and the number of natural flora, reducing the viscosity of digestive substances, enhancing the immune system, and lowering the blood cholesterol level (Ritz et al., 1995).

Milk thistle plant or *Silybum marianum* L. Gaernt. (*S. marianum*), sometimes called wild artichoke, is a member of *Asteraceae* family (Pepping, 1999). It has been previously used in medicine as a natural remedy for the liver and biliary tract (Morazzoni and Bombardelli, 1995). The dried extracts of *S. marianum* seeds contain approximately 60% silymarin (Bhattacharya, 2011). It is the active ingredient of milk thistle, which represents 4% of the dried seeds or in the aerial parts of the plant (Rajiha, 2012). It can be used as a non-toxic, safe, and cheap liver tonic feed additive to substitute synthetic drugs in poultry diets (Saeed et al., 2017). Silymarin was initially found in the Mediterranean mountain, North Africa, and Asia, but today, it has been grown in many parts world-wide (Khan et al., 2009).

Silymarin contains many flavonolignans such as silybin (50%-60%), silychristin (20%), silydianin (10%), and isosilibine (5%) as well as flavonoid (taxifolin) (Federico et al., 2017; Attia et al., 2019). Silymarin complex showed antioxidant, anti-inflammatory, anti-fibrotic, anti-lipid peroxidative, immune stimulant, and hepatic cells stabilizing effects (Suchy et al., 2008; Saeed et al., 2017). Furthermore, milk thistle seeds contain betaine, trimethyl glycine, and essential fatty acids which involved in the hepatoprotective and anti-inflammatory actions of the silymarin complex (Saller et al., 2001).

The different beneficial effects of silymarin have been previously reported in poultry production. It is considered as a potential feed additive to broilers in terms of enhancement of the growth performance, prevention of oxidative stress, improvement of the meat quality, increasing the production of polyunsaturated fatty acids, and stimulation of immune status (Zaker-Esteghamati et al., 2020; Bagno et al., 2021; Armanini et al., 2021).

Based on abovementioned findings, this review article aims to show the different potential uses of milk thistle derivative, silymarin, in poultry production system regarding its effect on production performance, antioxidant status, carcass traits, immune response, and intestinal health.



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## THE DIFFERENT EFFECTS OF SILYMARIN IN POULTRY PRODUCTION SYSTEM

### Production performance

Researchers showed that silymarin can improve the productive and reproductive performances and the health status of chickens (Abdulwahid and Oleiwi, 2021; Armanini et al., 2021), ducks (Egresi et al., 2020), and quails (Soto et al., 2003; Behboodi et al., 2017; Khaleghipour et al., 2019, 2020). Treatment of turkeys with silymarin at doses of 0.5 kg and 1 kg/ ton of feed mixture induced higher live body weight compared with un-treated control group (Gawel et al., 2003). Zarei et al. (2016) inoculated 1 ml of two dilutions (100 and 200 mg/L) of milk thistle extract *in ovo* and then added it to the feed mixture at a dose of 100 mg/kg. The results indicated higher final live weight of chickens compared to the control group. Besides, Abdalla et al. (2018) found an improvement in the body weight of chickens supplemented with silymarin (25 g/kg of diet) under the Egyptian summer conditions. Broiler chickens consumed silymarin (160 mg/kg diet) at the starter, grower, and finisher stages of rearing showed improvement of the body weight gain and feed conversion indices (Mousa and Osman, 2016). In the study of Shahsavan et al. (2021), dietary inclusion of 3%, 9%, and 12% of *S. marianum* oil extraction byproduct into the diet of broilers induced an increase in the body weight, feed intake, and the feed conversion rate. Various concentrations of silymarin (0, 100, and 200 mg/kg) in broilers enhanced the growth performance after exposure to lead-induced oxidative stress (Ebrahimi et al., 2013). In addition, it can alleviate the toxic effects of mycotoxins and improve the impairment of growth performance parameters elicited by mycotoxins in broilers (Kalorey et al., 2005; Chand et al., 2011; Surai, 2015; Morovat et al., 2016; Alhidary et al., 2017). Improved feed intake and feed conversion rate were observed in broiler chicks fed on a diet containing 0.8 mg/kg aflatoxin and treated with 600 mg/kg silymarin (Tedesco et al., 2004). Muhammad et al. (2012) reported that silymarin at a level of 10 g/kg diet increased the feed intake and the weight gain of broilers fed on rations contaminated with aflatoxin. In the same line, dietary supplementation with silymarin ameliorated the decreased feed intake and the body weight gain and improved the feed conversion rate of aflatoxin-challenged broiler chicks (Jahanian et al., 2017). A concentration of silymarin (500 g/ton feed) was able to mitigate the negative effect of aflatoxins on the metabolism and growth performance of laying Japanese quails (Sakamoto et al., 2018). Silymarin supplementation of aflatoxicated chicks increased the body weight gain as the result of increasing the feed intake and the protein synthesis in the hepatic cells (Sonnebichler and Zetl, 1986) as well as enhancing the digestibility and absorption of nutrients by increasing the digestive enzymes (Sultan et al., 2018). Moreover, enhanced growth performance parameters in silymarin-treated birds may be owing to the hepatoprotective and the detoxifying activities of *S. marianum* against mycotoxins (Baer-Dubowska et al., 1998; Fraschini et al., 2002). This compound can reduce the intestinal ulcer index and increased the mucin content (Huilgol and Jamadar, 2013).

### Antioxidant

Silymarin restored the oxidant and antioxidant activities to the normal physiological conditions that benefit animal health and consequently human consumers (Armanini et al., 2021). This compound restored all changes in liver and serum after intoxication with aflatoxin and that indicates its hepatotonic effect (Rastogi et al., 2000). Addition of 800 mg silymarin/kg feed to a diet of broilers containing 1 mg/kg aflatoxin inhibited the increase in alanine aminotransferase (ALT) activity (Jamshidi et al., 2007). Similar result was also reported by Tedesco et al. (2004) and Fani Makki et al. (2014). A recent study of Tsiouris et al. (2021) indicated that dietary supplementation of broiler diet with detoxifying agent containing modified zeolite, *Bacillus (B.) subtilis*, *B. licheniformis*, *Saccharomyces cerevisiae* cell walls, and silymarin ameliorated the adverse effects aflatoxin and ochratoxin. In ducks, a concentration of 0.5% silymarin decreased the oxidative stress of the liver after feeding on diets containing zearalenone and deoxynivalenol (Egresi et al., 2020). In other avian species such as Japanese quails, silymarin reduced the concentration of triglyceride and cholesterol when compared with carbon tetrachloride treated group (Behboodi et al., 2017; Moradi et al., 2017). Silybin, the major active constituent of silymarin, has antioxidant characters, hepatoprotective effect, and free radical scavenging activities (Fraschini et al., 2002; MacDonald-Ramos et al., 2021). This antioxidant effect may achieved by reservation of the hepatocytes membranes integrity, stabilization of phospholipid structure, activation of nucleic acids and protein biosynthesis, and stimulation of immunity (Vargas-Mendoza et al., 2014; Saeed et al., 2017). Silymarin decreased the secretion of some hepatic enzymes such as ALT, aspartate aminotransferase (AST), and alkaline phosphatase into blood as a result of hepatic injuries from free radicals (Amiridumari et al., 2013; Armanini et al., 2021) and also reduced the oxidation of lipid and protein (Alhidary et al., 2017) and the apoptosis of DNA (Upadhyay et al., 2010). Moreover, it has been found that silymarin prevented lipid peroxidation and returned some antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase in the hepatic cells of chickens after ochratoxin damaging effects (Yu et al., 2018; Armanini et al., 2021). This compound also returned some other antioxidants vitamins such as vitamins E and vitamin C in the liver (Pradeep et al., 2007). In a quail's trial, the results indicated that silymarin decreased the levels of bilirubin, malondialdehyde (MDA), ALT, triglyceride, and cholesterol, while increased the levels of albumin, protein total, superoxide dismtase, total antioxidant, and glutathione peroxidase (Moradi et al., 2017). Alassi and Allaw (2020) found that addition 1 g/kg milk thistle seed powder in quail's diet lowered the level of cholesterol, glutathione, MDA, ALT, and AST. Silymarin reduced the biliary cholesterol and phospholipids which may be in part due to decreased liver cholesterol synthesis (Crocenzi and Roma, 2006; Bhattachrya, 2011). Silymarin may be able to mitigate the oxidative stress-induced



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by carbon tetrachloride in broilers through modulation of oxidative stress biomarkers and hepatic oxidative genes expression (Baradaran et al., 2019).

Bhattachrya (2011) found that silymarin may maintain the normal renal function and silibinin can reduce the oxidative damage to kidney cells *in vitro*. In the same line, silymarin (259 µM) affected heat shock protein expression and prevented its alleviation by heat stress on chicken lymphocytes cells (Oskoueian et al., 2014). The previous study also showed that silymarin was able to normalize the expression of biomarkers such as MDA, tumor necrotizing factor-like, interferon (IFN-γ), and interleukin (IL-1β) genes in heat-induced chicken hepatocytes. Moreover, the study of Ledur and Santurio (2020) indicated that the *in vitro* addition of 5 µM silymarin to PK-15 cells exposed to different mycotoxins reduced the reactive oxygen species formation.

Silymarin may exert the hypoglycaemic effect through increasing the secretion of insulin via beta cells of the pancreas, enhancing the renovation of pancreatic cells, and protection of the pancreatic tissues against some metabolic damage (Soto et al., 2004; Kshirsagar et al., 2013). In addition, silymarin could regulate the liver enzymes involved in metabolism of carbohydrates causing reduction in the blood glucose level and restoring weights. This occurs due to decrease the activity of liver phosphorylase activity and increase glucokinase and glycogen synthase (Abascal and Yarnell, 2003).

**Carcass traits**

The highest breast weight muscle was detected in broilers received 1% of silymarin, compared with the groups consumed the different levels of aflatoxin (Chand et al., 2011). Addition of both L-carnitine and silymarin at levels of 300 mg and 160 mg/ kg diet, respectively reduced the abdominal fat deposition and increased the weight of thigh muscles of broiler chickens in comparison with the control group (Mousa and Osman, 2016). Zaker-Esteghamati et al. (2020) concluded that dietary addition of 4-15% silymarin improved the sensory and qualitative properties of broilers meat after exposure to aflatoxin diet. The authors found that the highest carcass weight and breast weight were detected in chickens fed on 3% silymarin oil. The improvement of carcass yield of silymarin-supplemented broilers may be related to the increase in protein synthesis (Sonnebichler and Zetl, 1986; Gawel et al., 2003; Jahanian et al., 2017). It has been found that silymarin has a similar structure to the steroid hormones, accordingly, it can pass to the nucleus and improve the formation of ribosomes via increasing the synthesis of structural and functional proteins by acting on rRNA enzymes (Negahdary et al., 2015).

It can enhance the meat polyunsaturated fatty acid profile which impaired by mycotoxin (Armanini et al., 2021). Schiavone et al. (2007) found that silymarin decreased the lipid contents of the thigh and breast muscles of broilers and increased the muscle resistance to oxidative stress. Changes in fatty acids metabolism at the hepatic level is owing to the hepatotonic effect of this compound (Saeed et al., 2017).

**Immune response**

Regulation of the immune system induced by silymarin depends on the method used and its concentration (Gharagozloo et al., 2010). Improvement of the immune status after feeding on milk thistle fruits was reported (Thyagarajan et al., 2002; Khariv et al., 2017; Alassi and Allaw, 2020; Bagno et al., 2021). Early study of Basaga et al. (1997) showed that milk thistle could enhance the immune system via its powerful antioxidant and free radical scavenging action. Long term administration of silymarin could improve the immune response by increasing the production of T-lymphocytes and IL and also it could be useful as a therapeutic adjuvant for autoimmune and infectious diseases (Das et al., 2008). Saeed et al. (2017) demonstrated that silymarin can modulate the immune response of birds by increasing the levels of IL-4, IL-10, and IFN-γ. Some studies indicated that the use of silymarin under oxidative stress of carbon tetrachloride can have a positive effect on the humoral immunity of Japanese quail via increasing in the concentrations of immunoglobulin G, the total antibodies, and white blood cell count (Moradi et al., 2017). Vitamin E and silymarin alone or in combination improved the immunotoxic effects induced by ochratoxin in Leghorn cockerels (Khatoon et al., 2013). Silymarin, as antioxidant, has protective action against the oxidative damages on the immune organs such as bursa of Fabricious, thymus, and spleen (Chand et al., 2011). This product decreased the relative weights of bursa of Fabricious and spleen, while increased the relative weight of the thymus (Moradi et al., 2017). Similarly, *S. marianum* was efficient in protection of spleen and bursa of Fabricious against the adverse effects of aflatoxin (Kalorey et al., 2005; Fani Makki et al., 2013). Chickens fed by diets containing 9% of *S. marianum* oil extraction byproduct showed greater spleen weights compared to chickens fed concentrations of 3%, 6%, and 12% (Shahsavan et al., 2021). A recent study of Bagno et al. (2021) showed increasing the content of γ-globulins in the serum of chickens fed on various doses of milk thistle extract (0.1, 0.5, 1.0, 1.5, and 2.0 mg/kg of body weight) as compared to the control group. Dumari et al. (2014) demonstrated that the serum antibody titers against Newcastle and influenza diseases viruses were higher than those recorded in aflatoxin treated group. Lutensko et al. (2008) found that silymarin phytosome increased albumin and globulin levels when compared with aflatoxicated broiler chickens. The cutaneous basophilic hypersensitivity response to phytohemagglutinin-P injection indicated that chickens received 9% of *S. marianum* oil extraction byproduct recorded high wing web thickness at 24h following injection (Shahsavan et al., 2021). Denev et al. (2020) demonstrated an increase in the level of serum betalysine after ditary addition of silymarin to ochratoxin challenged broiler chickens.


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### Intestinal health

Treatment of Japanese quails with 1 ml/kg body weight silymarin increased the length of villi and the ratio of villi length to crypt depth (Moradi et al., 2017). Dietary supplementation of aflatoxicated broilers with 500 ppm silymarin increased the villi height and width, the ratio between villi height and crypt depth, and the apparent villi absorptive area (Jahanian et al., 2017). Accordingly, this product can protect the villi from endotoxins produced by pathogenic bacteria. Shahsavan et al. (2021) found an increase in the duodenum, jejunum, and cecum of broilers fed on *S. marianum* oil extraction byproduct at the levels of 6%, 9%, and 12% compared to control.

Both Gram-positive and Gram-negative bacteria can be affected by silymarin and silibinin (Lahlah et al., 2012). In other study, caecal population of *Lactobacillus*, coliform, *Escherichia coli* (*E. coli*), total aerobes, and *Lactobacilli*/*E. coli* ratio were not influenced by the treatments by *S. marianum* oil extraction byproduct (Shahsavan et al., 2021). Under the *in-vitro* conditions, *S. marianum* showed antibacterial activities against *Staphylococcus saprophyticus*, *E. coli*, and *Klebsiella pneumonia* (Evren and Yurtcu, 2015). Generally, silymarin exerts its antibacterial effect through hydroxyl group which binds with the bacterial membrane proteins leading to the leakage of vital components of the cells (Lee et al., 2003; Bessam and Mehdadi, 2014).

## CONCLUSION

Application of natural alternative to antibiotics in poultry production system is urgently needed. Phytobiotics gained a great acceptance as one of these alternatives. Silymarin, a derivative of milk thistle herb, is widely used as a potent hepatotonic and antioxidant natural feed additive. Silymarin has a potential to increase the production performance in broilers and layers, increase the antioxidant status, improve the carcass trait, stimulate the immune response, and enhance the intestinal health. Thus, it is recommended to use such compound as feed additive in poultry field.

## DECLARATIONS


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### Author's contribution

Abd El-Ghany WA has collected and drafted the manuscript, formatted it, and approved the final manuscript.

### Conflict of interests

The author has not declared any conflict of interest.

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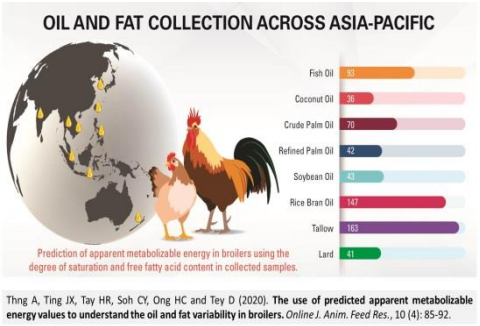

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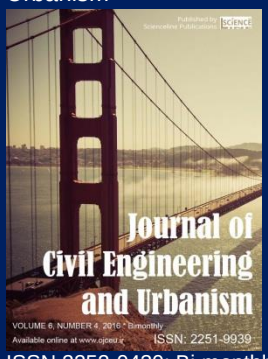


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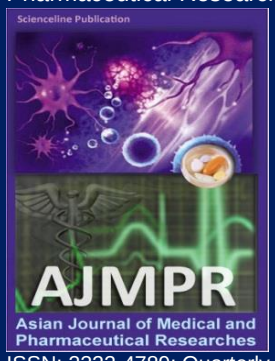


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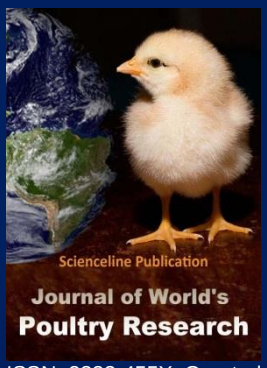


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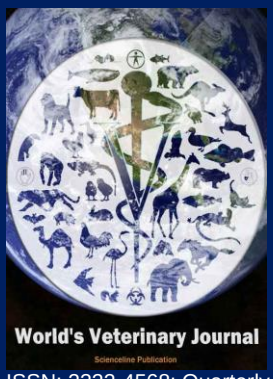


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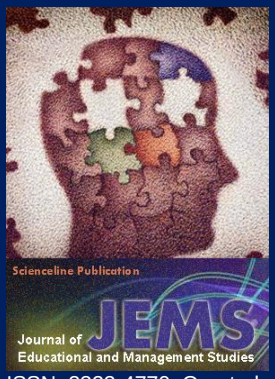


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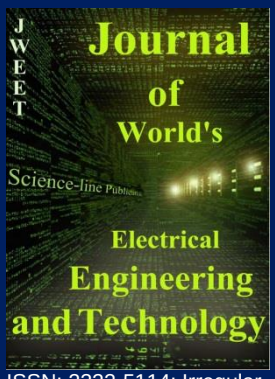


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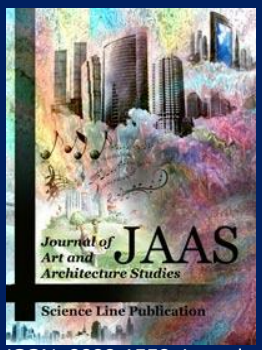


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