MODERN MEDICINE AND PRACTICE
https://www.innovatus.es/index.php/ejmmp
EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE
Vol. 2 No. 5 (2022) EJMMP ISSN: 2795-921X

EFFECT OF REPLACEMENT OF WHEAT (Triticum aestivum l) BRAN FOR COCOA (Theobroma cacao) BEAN SHELL ON THE GROWTH PERFORMANCE, SENSORY EVALUATION AND CARCASS CHARACTERISTICS OF JUVENILE CATFISH (Clarias gariepinus)

A. O. Ejelowoicho, A. Isaah, S. M. Tama, A. I. Ikposhi, A. I. Musawwir, Jolayemi, K.O and R. A. Hamzat

Department of Animal Science, University of Abuja, Abuja, Nigeria

Abstract: *The study was conducted to evaluate the effect of replacement of wheat (Triticum aestivum l) bran for cocoa (Theobroma cacao) bean shell on juvenile catfish (Clarias gariepinus). There were five treatments with T1 (basal diet without cocoa bean shell which served as the control), T2 (basal diet with 2.5% of cocoa bean shell), T3 (basal diet with 5.0% of cocoa bean shell), T4 (basal diet with 7.5% of cocoa bean shell), T5 (basal diet with 10% of cocoa bean shell), the experiment was conducted for 63 days. Data were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) of five treatments and each treatment replicated thrice. The chemical analysis of the feed showed a decreasing trend in crude protein across the treatments from T1 to T5 and an increasing trend in crude fibre from T1 to T5. There were significant differences ($p<0.05$) in the results of growth performance obtained from the experiment among the treatments except for the total feed intake ($p>0.05$). The organoleptic results for each treatments shows that: T2 has the best color with a mean of 8.16, T3 has the best taste with a mean of 8.10, T5 has the best texture with a mean of 8.32, T3 has the best smell with a mean of 8.00, T5 has the best appearance with a mean of 8.45 and T3 has the best overall acceptability with a mean of 8.26, values obtained for mean weekly body length increment, mean weekly body width increment and mean weekly head and tail length were not affected by inclusion of CBS in the diets of catfish. The values obtained for carcass characteristics of catfish were not significantly different ($P > 0.05$) and so not affected by the treatments. Cocoa bean shell could be included in the diet of catfish up to 2.5 % inclusion rate without causing any deleterious effect on the growth of the fish.*

Keywords: *Cocoa bean shell, Performance, Juveniles, Theobromin, Wheat bran*

INTRODUCTION

Fish is an important and the cheapest source of animal protein and account for about 37% of Nigeria total protein requires (FAO, 2002). Fish provides approximately 16% of the animal protein consumed by the world population (FAO, 1997). It is particularly an important protein source in require where livestock is relatively scarce.

Billions of people mostly in developing countries depend on fish as a primary source of animal protein (FAO, 2000). FAO estimated that by the year 2010, demand of fish will increase by 13.5%-18.5% or to about 105-110% million metric tons (FAO, 2000). Further increase in capture fisheries are not anticipated

under the current global condition (Dunham et al., 2001). Faturoti (1999) noted that recent trends all over the world pointed to a cleared in landing from capture fisheries which are all indicator that fish stock have approached or even exceeded point of maximum sustainable yield. The food and agriculture organization recommended that an individual takes 3 series per capture of animal protein per day for sustainable growth and development. However, the animal protein consumption in Nigeria is less than 8g per person per day which is a far cry from the FAO polonium recommendation. The rapid growth of Nigeria population has led to insufficiency in supply of animal protein source food consequently also led to tremendous efforts resulting in increasing animal production. Fish is a major source of animal protein source and an essential food item in diet of many people in Nigeria. Fish is also a good source of Thiamine, Riboflavin, Vitamin A and D, Phosphorus, Calcium, and Iron. It is also very high in polyunsaturated fatty acids which are important in lowering blood dialectal level, it is therefore suitable for complementing high carbohydrate diets typical of low-income group in Nigeria (Areola, 2008; Alagbe, 2019). Apart from being food, fish is also an important source of income to many people in developing countries including Nigeria. FAO (1996) confirms that as much as 5% of the African population have 35million people depending wholly or partly on fisheries sector for their livelihood.

Catfish of the family *Claridae* comprise the most commonly cultivated fishes in Nigeria, the growth of aquaculture in Nigeria is now largely being boosted by a steady rise in catfish culture, inadequate availability of seed stocking and feed use to be major problems. The favoured catfish species in Nigeria aquaculture includes *Clarias gariepinus, Heterobranchus bidorsalis* and *Clarias nigrodigitatus. Heterobranchus* species is the more commonly cultured fish in the South eastern parts of Nigeria. Africa catfish is popular in the market and has great potentials to boost the rapidly growing Nigeria aquaculture. *Clarias gariepinus* is generally considered to be one of the most important tropical fresh water fish species for aquaculture whose aquaculture potential have been documented (Dada and Wonah, 2003). Bruton (1979) pointed out that *C.gariepinus* has also high fecundity rate, grows faster, tolerates high density and environmental extremes. It also accepts wide range of natural and artificial food and adapts to a variety of feeding modes in expanded niches.

Cocoa (*Theobroma cacao L.*) bean shell, a byproduct of the chocolate industry, is usually removed away from the bean using winnowing machine and sold to fertilizer companies or own use as fertilizer. Although the shell contains nutrients that benefit the body, for examples, polyphenols (ca. 1–2%), alkaloids such as theobromine (ca. 1–2%), vitamins such as Vitamin D, minerals such as calcium and phosphorus, amino acids, as well as soluble and insoluble dietary fibers (ca. 25–30%), etc., the utilization to upgrade its value is still low. In fact, the shell covers almost seventeen percent of total cocoa bean weight. In 2017, the world production of cocoa reached 4.7 million tons, increased from 4.0 million tons in 2016 (International Cocoa Organization (ICO) 2017).

Cocoa shell comprises of seed coat and embryo. The shell is a dry, crisp, slightly fibrous brown husk with a pleasant odor resembling that of chocolate. When the shell is removed, it may contain 2–3% of an unseparated cocoa nib. Cocoa shell is a good source of energy and minerals, P and Mg for ruminants. The fiber content is equivalent to medium quality grass hay in feeding value. The chemical composition of cocoa bean shell indicates it might be a useful ingredient for ruminant feeding. (Meffeja et al 2006) presented crude protein values of 5.9% and crude fiber of 21.3% (Marcel et al). asserted that cocoa bean shell contains 17.6% crude protein, 4.6% fat, 0.36% Ca, 0.61% P, 0.06% Na, U 61% Mg and 1.6% theobromine.

Flachowsky concluded that cacao bean shells may be used as roughages in ruminant diets up to 5% of dry matter intake. The factor limiting the use of cocoa bean shell in feed is the theobromine level which is dependent on the way the cacao bean is prepared for the market. Originally the shell contains a limited amount of theobromine acquired from the nib during fermentation. The shell of most well-fermented

commercial cacao beans contains over 1% theobromine five samples contained between 0.80 and 1.69%. Abiola reported a level of 1.9% presented a complete analysis of a commercial sample of roasted shells; the average theobromine content was 13 g/kg (8.0–16.9 g/kg), and the caffeine content around 1 g/kg.

The nutritive value of cocoa shell was also studied in vitro and nylon bag technique and a feeding trial was carried out in growing cattle, the dry matter digestibility of cocoa shell was 63.5%. Approximately 30% of cocoa shell protein disappeared from the rumen after 12 h and there was a small increase after that time. Whereas for fat, there was an increasing amount that disappeared from the rumen after 12 h but reached the maximum value (73%) at 48 h. (Olayinka *et al*, July 13th 2019).

The aim of this experiment was to determine the effect of replacement of wheat (*Triticum aestivum l)* bran for cocoa *(Theobroma cacao)* bean shell on the growth performance, sensory evaluation and carcass characteristics of juvenile catfish *(Clarias gariepinus)*

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the University of Abuja, Teaching and Research Farm Gwagwalada located between latitudes $8^{o}57^{1}$ and $8^{o}55^{1}$N and longitude $7^{o}05^{1}$ and $7^{o}06^{1}$E (Balogun, 2001).

Collection and preparation of cocoa bean shell

Fresh Cocoa bean shell was collected from Ile Oluji, Ondo State, Nigeria. It was identified and authenticated at the Department of Crop Science, University of Abuja, Nigeria. The shell was sundried until a constant weight was obtained. Thereafter, it was milled separately using laboratory grinder and stored in a labeled container for further analysis.

Animals and their management

A total of number 300 juvenile catfish were used for the feeding trial that lasted 63days in a completely randomized design. The fish with an average weight of 11.50 ± 0.70 was purchased from a reputable farm in Ibadan, Oyo State, Nigeria. The trial has 5 treatments and was replicated thrice with 15 fish per replicate. Five diets were formulated which contained 0.0, 2.5, 5.0, 7.5, 10.0 % cocoa bean shell and were designated T1, T2, T3, T4, and T5 respectively (Table 1). The fish were reared in a 65liters bowl and were kept in a shade of 8m by 4m, the top of the bowl was covered with mosquito net to prevent predators. The experimental diet was supplied in *ad libitum.* The fish in the respective bowls were fed twice a day with 33g of the basal diet with the use of a sensitive weighing scale, the weight gain was taken on a weekly basis with the sue of sensitive weighing balance.

Data collection

The head length and width, full length, tail length and width were taken with use of meter rule. At the end of the feeding trial 5 fish was taken from each treatment for carcass analysis. The dressing percentage was calculated as the ratio of carcass weight to live weight. Sensory evaluation was carried out by a panel of 15 members from faculty of Agriculture according to the method of Eyo (2001). The panelists were trained and given water for washing their mouth before testing to avoid bias. Questionnaires were used by evaluators and scoring was done to determine the most preferred/acceptable sample. Evaluation was done on hedonic scale of 9 based on the method of Doe and Olley, (1990 as cited by Oyero 2006) A Hedonic scale as reported by (Oyero 2006) was used as follows; 9- like extremely; 8- like very much; 7- like moderately; 6- like slightly; 5- neither like nor dislike; 4- dislike slightly; 3- dislike moderately; 2- dislike very much; 1- dislike extremely. Parameters tested for include; color, smell, taste, texture, appearance and overall acceptability.

Data analysis

All data obtained were subject to one-way ANOVA using SPSS (23). Significant means were separated using the Duncan multiple range test where ($p \leq 0.05$)

Table 1. Gross Composition of Experimental Diets of Juvenile Catfish (*Clarias gariepinus*)

TREATMENT AND % TEST MATERIAL					
INGREDIENTS (Kg)	**T_1**	**T_2 (2.5%)**	**T_3 (5%)**	**T_4 (7.5%)**	**T_5 (10%)**
Cocoa bean shell (CBS)	00.00	00.50	01.00	01.50	02.00
Maize bran	23.00	23.00	23.00	23.00	23.00
Wheat bran	20.00	19.50	19.00	18.50	18.00
Soya bean meal	12.00	12.00	12.00	12.00	12.00
Groundnut cake	25.00	25.00	25.00	25.00	25.00
Fish meal local	15.00	15.00	15.00	15.00	15.00
Rice bran	03.20	03.20	03.20	03.20	03.20
Salt	00.25	00.25	00.25	00.25	00.25
Vitamin Premix	00.25	00.25	00.25	00.25	00.25
Methionine	00.15	00.15	00.15	00.15	00.15
Lysine	00.15	00.15	00.15	00.15	00.15
Palm oil	01.00	01.00	01.00	01.00	01.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Calculated analysis

Dry matter	93.00	91.00	93.09	92.00	90.00
Moisture	7.00	9.00	6.01	8.00	10.00
Crude protein	34.82	32.03	31.88	31.03	30.09
Crude fibre	13.08	14.15	14.88	15.03	15.05
Ether extract	8.87	6.60	6.00	5.64	5.50
Ash	11.44	12.03	12.55	12.94	13.00
NFE	37.87	40.34	45.56	42.39	41.41
Energy kcal/kg	2503.1	2500.8	2499.4	2490.5	2480.3

NFE: Nitrogen Free Extract

RESULTS AND DISCUSSION

Phytochemical composition of Cocoa bean shell

The air-dried cocoa bean shell contained some phytochemicals. Result from this study reveals the presence of favonoids (10.74 %), alkaloids (4.06 %), tannins (6.52 %), terpenoids (7.11 %), phenols (17.22 %), saponins (3.29 %), oxalates (0.44 %) and theobromine (22.64 %). Phytochemicals are non-nutritive plant chemicals which plays specific pharmacological effects in animal health (Singh et al., 2021). They also perform multiple biological activities such as antibacterial, antifungal, antioxidants, hypolipidemic, and analgesic (Adewale et al., 2021; Shittu et al., 2021). Phenols are antioxidants capable of scavenging free radicals thus preventing diseases (Musa et al., 2020). Adisa et al. (2010); Shittu and Alagbe (2020) reported that tannins are known to possess antibacterial and antiviral activity. Terpenoids has high therapeutic value and function as antimicrobial, anticarcinogenic and anti-diuretic (Olafadehan et al., 2020). Flavonoids are known to posses antibacterial properties (Oluwafemi et al., 2020). Phenolic and flavonoid contents and

total antioxidant capacities of cocoa are higher than that of other phytochemical-rich foods (Giovanni et al., 2014).The tricyclic structure of the flavonoids determines their antioxidant effects; phenolic quinoid tautomerism and the delocalization of electrons over the aromatic system scavenge free radicals (Schroeter et al., 2006). Theobromine has been shown to possess high bioavailability and is capable of stimulating the heart muscle, relaxes bronchial smooth muscles in the lungs and plays an important role in the transmission of intracellular signals (Sprügel et al., 1977; Neufingerl et al., 2013).

Table 1: Phytochemical composition of cocoa bean shell

Parameters	Composition (%)
Flavonoids	10.74
Alkaloids	4.06
Tannins	6.52
Terpenoids	7.11
Phenols	17.22
Saponins	3.29
Oxalates	0.44
Theobromine	22.64

Table 3 reveals the growth performance of Juvenile catfish. The final body weight (FBW), weight gain (WG), average daily weight gain (ADWG), feed intake (FI) and feed conversion ratio (FCR) ranged from (24.18 - 31.70 g), (16.00 - 17.04 g), (0.19 - 0.21 g), (334.5 - 341.80 g) and (1.63 - 1.78) respectively. There was no significant difference (p>0.05) in total feed intake among the treatments. However, weight gain, average daily feed intake and feed conversion ration were significantly ($p<0.05$) influenced by the treatments. Treatment 2 (T2) fed 2.5 % cocoa bean shell (CBS) had the highest weight gain compared to the other treatments. This could imply that the fish were able to tolerate the test material at 2.5 % without causing any negative effect on their growth. It was observed that the highest replacement of wheat bran with 10 % CBS affected the growth of the fish in treatment (T5). The positive response in growth recorded among fish in T2 could be attributed to the presence of bioactive chemicals in CBS especially theobromin which have been reported to perform multiple biological activities in the body of animals. The results obtained in this current study are in agreement with the findings of Omovwohwovie et al. (2018) when melon shell was used as a replacement for maize.

Table 3: Performance Characteristics of Catfish fed Experimental diet

PARAMETERS	**T1 (0% CBS)**	**T2 (2.5% CBS)**	**T3 (5% CBS)**	**T4 (7.5% CBS)**	**T5 (10% CBS)**	**SEM**
IBW (g)	11.67	11.73	11.53	11.80	11.58	0.09
FBW (g)	28.72^{b}	31.70^{a}	27.76^{b}	24.18^{b}	28.37^{b}	1.83
WG (g)	13.04^{b}	16.38^{a}	16.22^{a}	16.08^{a}	16.00^{a}	1.81
ADWG (g)	0.17^{b}	0.26^{a}	0.24^{a}	0.20^{a}	0.19^{b}	0.03
FI (g)	340.4	337.62	330.9	334.5	341.8	36.01
FCR	1.71^{b}	1.63^{a}	1.74^{b}	1.76^{b}	1.78^{b}	3.79

IBW; initial body weight, **FBW**; final body weight, **WG**; weight gain, **ADWG**; average daily weight gain, **FI**; feed intake, **FCR**; feed conversion ratio

SEM = Standard error of mean

CBS = Cocoa bean shell

The sensory evaluation of catfish fed the experimental diet at different inclusion level of cocoa bean shell is shown in Table 3. The parameters evaluated includes colour, taste. texture, smell, appearance and acceptability respectively, which ranges from (7.97 – 8.16 %), (7.81 - 8.10 %), (7.40 - 8.32 %), (7.65 - 8.00 %), (8.19 - 8.45 %), (8.10 - 8.26 %). The results obtained shows that all the parameters evaluated (colour, taste, texture, smell, appearance and acceptability) were not significantly affected ($P<0.05$) by the dietary treatments. **(Table 4)** However, the dressing percentage was similar across the treatment hence the inclusion of CBS in the diets of catfish did not alter the dressing percentage of fish negatively. The nutrients obtained by catfish for the production of flesh in all the CBS based diets were adequate since there was no depression in the dressing percentage.

Table 3 Sensory Evaluation of Catfish Fed Graded levels of Cocoa Bean Shell

Parameters	T1	T2	T3	T4	T5	SEM
Colour	8.10	8.16	8.09	8.00	7.97	0.62
Taste	8.07	8.00	8.10	7.94	7.81	0.59
Texture	7.74	7.93	7.97	8.06	8.32	0.56
Smell	7.84	7.65	8.00	7.97	7.94	0.60
Appearance	8.39	8.19	8.23	8.32	8.45	0.50
Acceptability	8.26	8.16	8.19	8.10	8.16	0.67

Table 4 Carcass Analysis of Catfish Fed Graded levels of Cocoa Bean Shell

	T1	T2	T3	T4	T5	SEM
LW(g)	35.66	43.33	36.33	33.00	35.33	2.89
DBW(g)	32.66	38.33	35.00	30.66	31.33	2.65
DBP(%)	101.2	89.36	96.93	94.26	88.46	3.07
HDRW((g)	26.33	24.66	21.66	21-66	20.00	1.85
HDP(%)	75.56	57.06	56.80	69.63	56.16	3.48

LW: Live weight; DBW: Dress Body Weight; DBP: dressing Body percentage; HDRW: Headless dress Round weight; HDP: Headless Dressing Percentage

CONCLUSION

Cocoa bean shell is rich in several nutrients and bioactive chemicals that exhibit pharmacological effects which could also be used to bridge the gap between food safety and fish production. The outcome of this study shows that cocoa bean shell could partially replace wheat brown up to 2.5 % inclusion rate in the diet of catfish without any negative impact on its general performance.

REFERENCES

1. Abiola, S.S. and Tewe, O.O. (1991). *Chemical evaluation of cocoa byprodukts. Trop. Gric. (Trinidad) 64: 335-336.*
2. Bruton, N.M (1979) the breeding biology and early development of Clarias gariepinus in lake sibiya, South Africa with review of breed in species of subgenus clarias : trans 200. Sci. London 35. Pp 1-45
3. Dada A.A and Wonah, C. (2003) production of exotic Clarias gariepinus at varying stocking density in outdoor ponds. Journal of aquatic science 18(i): pp21-24
4. Dunham, R.A., Majumdar, K., Hallerman, E., Bartley, D., Mair, G., Hulata, G., Liu, Z., Pongthana, N., Bakos, J., Penman, D., Gupta, M., Rothlisberg, P. & Hoerstgen-Schwark, G. 2001.

5. Eyo A A (2001): Fish processing technology in the tropics. A publication of National Institute for Freshwater Fisheries Research (NIFFR) PMB 600, New Bussa, Nigeria.
6. FAO (1987). Nutrition and feeding of farmed fish and shrimp a training named food and agriculture organization of the United Nations, Brazil. June 1989
7. FAO (1996). Food for all poor issued on the occasion of the world food summit in rome, FAO Rome, p 64.
8. FAO (2000) The State of World Fisheries and Aquaculture 2000. FAO, Rome, Italy.
9. FAO (2001), *The state of Food Insecurity in the World 2001 Rome*.
10. FAO, (1997). Review of the state of world agriculture, FAO fisheries circular. Pp886:15,Rome, Italy.
11. FAO, (2002). Projected population and fish demand and supply in Nigeria.
12. Faturoti, E.O (1999) fisheries potential and investment opportunities in Nigeria paper presented at the fisheries society for Nigeria (FISCON), investors workshop half at the NIOMR Lagos April, 22, 1999.
13. Flachowsky G, Ivanowitsch Ochrimenko W, Koch H, Leopold G. Utilization of cacao bean shells as ruminants feeds. Beiträge zur Tropischen Landwirtschaft und Veterinärmedizin. 1990;28:93-99
14. Hamzat, R.A. and Adeola, O. (2011). Chemical evaluation of co-products of cocoa and kola as livestock feeding stuffs. J. Anim. Sci. Adv., 1: 61-66
15. International Cocoa Organization (ICO) (2017) Cocoa market review August 2017. Cote d'Ivoire
16. Meffeja F, Njifutié N, Manjeli Y, Dongmo T, Tchakounté J, Fombad RB. 2006. Digestibilité et influence des rations contenant des niveaux croissants de coques de cacao sur les performances des porcs en croissance finition. Livestock Research for Rural Development 18, 111.
17. Olayinka J.M, Sunday A.O, Emmanuel O, Akeem B.S, Solomon O.A, Elaigwu S. (July 13th 2019). *Exploration of Cocoa (Theobroma cacao) By - Products as Valuable Potential Resources in Livestock Feeds and Feeding Systems.* London: Intechopen.com/chapters/68088.
18. Alagbe, J.O. (2019). Role of *Moringa olifera* leaf meal on the growth performance of Poultry/African catfish – A review. *International Journal of Advanced Biological and Biomedical Research*. 7(3):249-257.
19. Adewale, A.O., Alagbe, J.O., Adeoye, Adekemi. O. (2021). Dietary Supplementation of *Rauvolfia Vomitoria* Root Extract as A Phytogenic Feed Additive in Growing Rabbit Diets: Haematology and serum biochemical indices. *International Journal of Orange Technologies*, 3(3): 1-12.
20. Schroeter, H.; Heiss, C.; Balzer, J.; Kleinbongard, P.; Keen, C.L.; Hollenberg, N.K.; Sies, H.; Kwik-Uribe, C.; Schmitz, H.H.; Kelm, M. (−)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proceedings National Academic Science USA 2006, 103, 1024–1029.
21. Singh, A.S., Alagbe, J.O., Sharma, S., Oluwafemi, R.A and Agubosi, O.C.P. (2021). Effect of dietary supplementation of melon (*Citrallus linatus*) seed oil on the growth performance and antioxidant status of growing rabbits. *Journal of Multidimensional Research and Reviews*, 2(1): 78-95.
22. Shittu, M.D., Alagbe, J.O., Adejumo, D.O., Ademola, S.G., Abiola, A.O., Samson, B.O and Ushie, F.T. (2021). Productive Performance, Caeca Microbial Population and Immune-Modulatory Activity of Broiler Chicks Fed Different Levels *Sida Acuta* Leaf Extract in Replacement of Antibiotics. *Bioinformatics and Proteomics Open Access Journal* 5(1): 000143.

23. **Musa, B.,** Alagbe, J.O., Adegbite Motunrade Betty, Omokore, E.A. (2020). Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture. *United Journal for Research and Technology*, 2(2):13-21.

24. Oluwafemi, R.A., Grace Funmi Reuben and Alagbe, J.O. (2020). Effect of feeding *Polyalthia longifolia* leaf meal as partial replacement of wheat offal. *European Journal of Biotechnology and Bioscience.* 8(4): 8-12.

25. Shittu, M.D and Alagbe, J.O. (2020). Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. *International Journal of Integrated Education*. 3(11): 119-124

26. Olafadehan, O.A., Oluwafemi, R.A and Alagbe, J.O. (2020). Carcass quality, nutrient retention and caeca microbial population of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. *Journal of Drug Discovery*. 14(33):146-154.

27. Sprügel, W.; Mitznegg, P.; Heim, F. (1977). The influence of caffeine and theobromine on locomotive activity and the brain cGMP/cAMP ratio in white mice. Biochemical Pharmacology 26: 1723–1724

28. Neufingerl, N.; Zebregs, Y.E.; Schuring, E.A.; Trautwein, E.A. (2013). Effect of cocoa and theobromine consumption on serum HDL-cholesterol concentrations: A randomized controlled trial. American Journal of Clinical Nutrition 97: 1201–1209.

29. Giovanni Scapagnini., Sergio Davinelli ., Laura Di Renzo. , Antonino De Lorenzo. , Hector Hugo Olarte., Giuseppe Micalii. , Arrigo F. Cicero and Salvador Gonzalez. (2014). Cocoa Bioactive Compounds: Significance and Potential for the Maintenance of Skin Health. Nutrients 6: 3202-3213.